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TECHNICAL REPORT
BL 28

(Revision of Special Report 211)

USE OF ULTRAVIOLET RADIATION IN MICROBIOLOGICAL LABORATORIES

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NOVEMBER 1960

U.S. ARMY CHEMICAL CORPS
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U.S. ARMY CHEMICAL CORPS RESEARCH AND DEVELOPMENT COMMAND
U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Maryland

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Safety Division
OFFICE OF THE SAFETY DIRECTOR

Project 4B11-05-015

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FOREWORD

The authors desire to recognize the guidance and encouragement given by Dr. A. G. Wedum throughout this project.

The services of Dr. Rudolph Nagy, who reviewed this report, were invaluable. Dr. Nagy made available valuable unpublished data which were included in this report and prepared several of the chapters. Mr. Richard H. Kruse assisted in editing the report and in preparing the Literature Cited and Indexes.

Many others contributed in a variety of ways toward making this report possible. Part of Chapter XI is the work of Mr. Herbert M. Decker and Mr. J. Bruce Harstad.

DIGEST

This report summarizes the results of a six-year research program which was established because it was desirable first to survey the literature concerning the action and use of UV radiation, and second, to determine experimentally the susceptibility of various types of microorganisms when exposed to radiation under conditions that might be found in the infectious disease laboratory. Finally it was planned to use the assembled data as a guide in developing, designing, and testing suitable UV installations for use in the infectious disease laboratory. Germicidal UV radiation is used in industry for protection of personnel and protection of the product. Its uses in this report have been directed primarily toward protection of personnel and test animals.

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I. INTRODUCTION

During the past several decades an enormous amount of experimental evidence has accumulated demonstrating the bactericidal, virucidal, and fungicidal properties of ultraviolet (UV) radiation. For the most part this has been a direct result of the development of new and better types of artificial UV sources. As a result of these data, the germicidal properties of UV radiation are well established and accurately defined, and the radiations are being used in many practical instances where the destruction of microorganisms is required. Ultraviolet radiation is also widely used as a research tool in the fields of cytochemistry and photochemistry. These latter uses, as well as its employment as a mutagen for a variety of purposes, will not be discussed in this report.

In safety programs for laboratories handling highly infectious materials, UV radiation was given early consideration. It seemed probable that the germicidal properties of radiant energy could be utilized, in conjunction with other control measures, to reduce the number of instances in which laboratory workers become infected with the microorganisms with which they work. It was also thought that the radiations could be used for controlling the common air-borne or dust-borne microorganisms which harass the bacteriologist by continually infiltrating sterile media and equipment.

Survey information presently available on the frequency of laboratory-acquired occupational illnesses in this country emphasizes the need which exists for various types of germicidal agents. The survey of Sulkin and Pike (278)* in 1951 listed 1342 laboratory-acquired infections, including diseases such as tularemia, psittacosis, tuberculosis, Q fever, and glanders. When the survey was brought up to date in 1956, (301) a total of 2262 laboratory infections were listed. It is believed that the reported infections represent only a fraction of those actually occurring because correct diagnosis is difficult in many instances, and there have been few attempts to discover inapparent infections.

The evaluation of many of the procedures and techniques used in infectious disease laboratories (237,238,244,288,300,319) and the realization of the hazards attending these techniques, particularly those involving air transmission of disease, also suggests the need for the use of aerogenic disinfectants such as UV radiation.

Several facts were noted early in the efforts to determine the utility of UV radiation in bacteriological laboratories. Much of the quantitative experimental data available were of little value when attempts were made to apply the information to practical use. The most obvious reasons for this were the lack of accurate measurements of UV intensity and variations in the UV sources used, in the methods of exposure and in the types of test organisms employed. In addition, various workers have not agreed on the effect

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* All such numbers in parentheses refer to applicable literature references; see Literature Cited, page 251.

of relative humidity and other physical factors affecting the biological reaction. It was evident that the radiations could be used in many different ways, and, for the most part, each use would require a specially designed installation. Other factors were encountered, such as the hazard of cutaneous or ocular burns from artificial UV sources, the effect of UV radiation on exposed equipment, and the effects of ozone.

The investigational program which is reported here was established because it was desirable first to survey the literature concerning the action and use of UV radiation, and second, to determine experimentally the susceptibility of various types of microorganisms when exposed to radiation under conditions that might be found in the infectious disease laboratory. Finally it was planned to use the assembled data as a guide in developing, designing, and testing suitable UV installations for use in the infectious disease laboratory.

Germicidal UV radiation is used in industry for protection of personnel and protection of the product. Its uses in this report have been directed primarily toward protection of personnel and test animals.

II. GENERAL CHARACTERISTICS OF UV

A. HISTORY

In 1878 two English scientists, Downs and Blunt (68), discovered that sunlight was bactericidal. This discovery led to the early pioneering on sunlight during the last decade of the 19th century (13,14,69,296,297). In 1892 it was suggested that a specific region of the sun's radiation was responsible for bactericidal effects (297). A Danish physician, Niels Rydberg Finsen, established the fact that two of the outstanding effects of sunlight, the bactericidal action, and the production of photo-erythema, resulted from invisible UV radiation. Many studies followed in which investigators studied sunlight to learn more about the bactericidal UV radiation. However, it was soon realized that the sun is a very unreliable source of radiation because the intensity varies with the time of day, the season of the year, and the elevation above sea level (25). The development of artificial UV radiation sources gave great impetus to studies of the germicidal activity of this radiation. The three principal types of artificial sources in use today as listed by Ellinger (74) are: (a) the arc lamps; carbon and mercury vapor; (b) the glow lamps, mercury or hydrogen discharge tubes; and (c) the spark lamps, such as the iron electrode where a discharge takes place between cold electrodes.

Radiation, whether visible or invisible, is an electromagnetic vibration. It is considered to be a wave or a quantum phenomenon. These waves are propagated at a speed of about 186,300 miles per second. Different types of light are defined according to the length of the wave (Figure 1). The radiant energy is in the form of photons, the energy per photon being a function of the frequency of the waves (oscillations per second). As the wave length becomes smaller, the energy value of the photon increases and the wave frequency increases. The energy state of UV radiation, as compared to infrared radiation, is responsible for the greater antimicrobial effects of the former. Radiant energy is absorbed by the organisms and used in the photo-decomposition of many of the essential compounds and enzymes. Some of the infrared radiation would be absorbed; however, this would not have the energy to rupture any chemical bonds. Monochromatic light, or radiation of one wave length, is rarely encountered except when obtained by special lamps or special prisms and filters. Consequently, it is customary to designate various regions in the radiant energy spectrum according to approximate maximum and minimum wave lengths.

The visible portion of the spectrum includes radiations whose wave lengths lie between about 4000 and 7700 angstrom units (4000A to 7700A). That portion generally designated as ultraviolet includes all the radiations from those overlapping the X rays (about 150A), to those bordering the visible. The spectrum is further divided on an arbitrary basis which depends upon the use for which the radiation is intended. Wave lengths shorter than 2000A have been called the Schumann region. The portion most

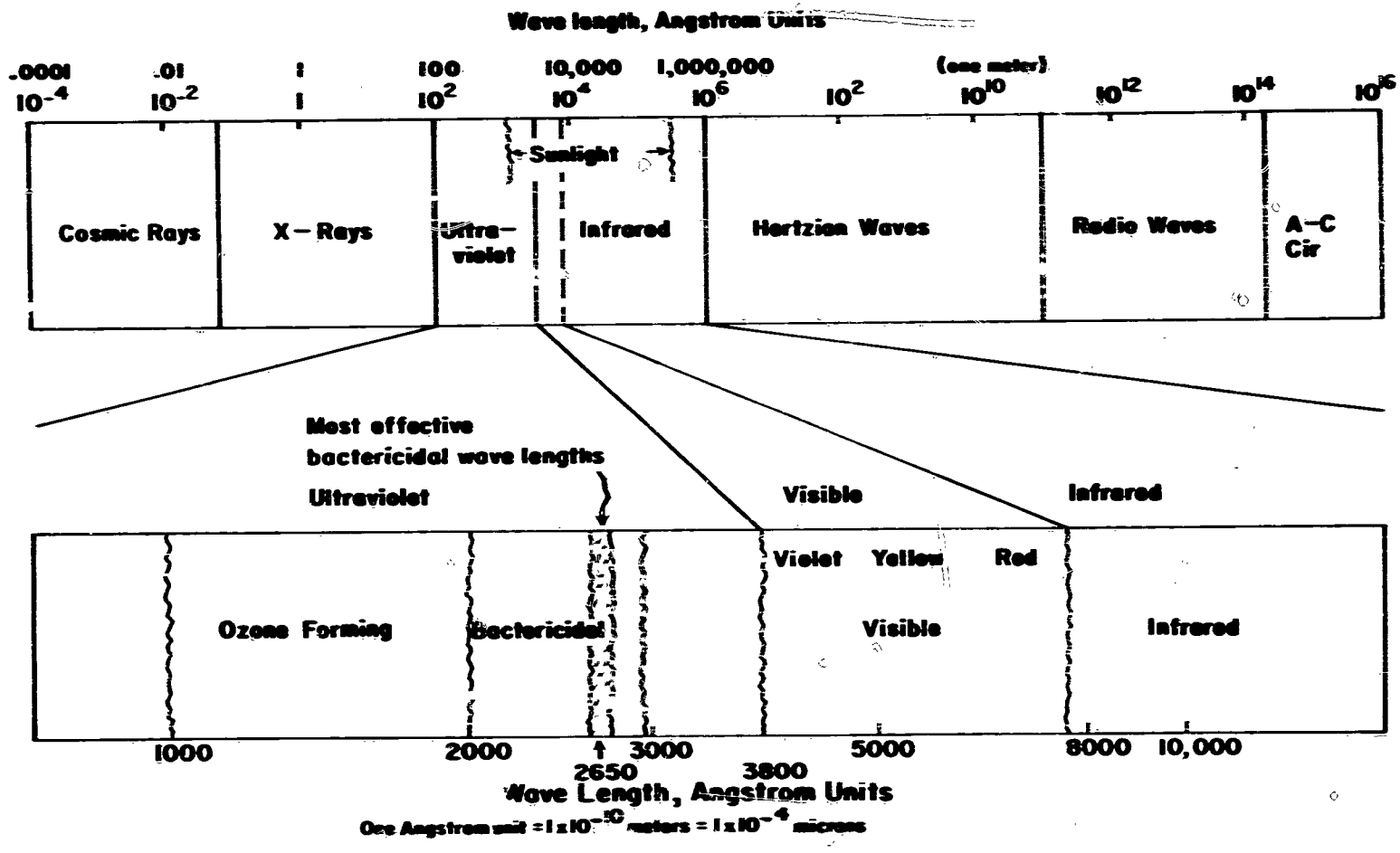

Figure 1. Spectrum of Radiant Energy.

commonly studied for bactericidal properties extends from 2000A to 3100A and is often called the abiotic region because these wave lengths kill or injure cells. Cellular destruction does not occur in the 3100A to 4000A range to the same extent as with the shorter radiation.

It is convenient to divide the entire UV spectrum into four general regions, based upon the use of the radiation. These four regions cannot be accurately defined because their effects tend to overlap the defined limit.

Region 1: 4000A to 3200A - This is the region nearest the visible and provides radiation which is used to produce fluorescent effects and photochemical reactions.

Region 2: 3200A to 2800A - Sometimes called biologically effective radiation. This radiation is antirachitic and aids in the production of vitamin D. Erythema and tanning is also produced.

Region 3: 3000A to 2000A - This region comprises the radiation which destroys bacteria, yeasts, viruses and molds.

Region 4: Below 2000A - This radiation is absorbed by the oxygen in the air and converts the latter into ozone. Radiation below 1000A will ionize air constituents.

B. MECHANISM OF BIOLOGICAL ACTION

Early attempts to explain the mechanism of the lethal action of UV radiation have been for the most part unsatisfactory. Bedford (23) supported the theory that organisms were killed by the action of hydrogen peroxide formed by the UV radiation. Moore and Webster (215) claimed that the germicidal action of sunlight was attributable to the formation of formaldehyde. It has been suggested by Voogd and Daams (294) that UV irradiation prevents multiplication of cells and allows normal dying to occur. This explanation does not satisfy the known action of UV radiation against many dormant spores.

Voluminous published experimental work has proved that, within the 4 general regions, various monochromatic wave lengths show decided tendencies toward exerting certain desired biological phenomena more efficiently. The so-called "action spectra" (100) relates the efficiencies which have been determined for certain ultraviolet effects by various wave lengths. Many data are available concerning the absorption of UV radiation by protoplasmic compounds. It has been found that UV radiation is more readily absorbed than other wave lengths by substances such as albumin and nucleic acid. Attempts have been made to correlate UV absorption by protoplasmic compounds with germicidal action, but, as pointed out by Giese (100), no case of perfect agreement has been found. A review of the action of UV radiation on protoplasmic compounds is beyond the scope of this report, but the information recorded in Table I, taken from data collected by Giese, serves to illustrate the correlation of some of the effects.

TABLE I. UV ACTION SPECTRA

UV Effects Which Resemble Absorption by:	
Nonconjugated proteins (albumin maximum absorption about 2800A)	Nucleic acid - maximum absorption about 2600A
(1) Effects on division of sea urchin eggs	(1) Mutagenic effects
(2) Immobilization of paramecia	(2) Virucidal effects
(3) Ciliary reversal & motility	(3) Bactericidal effects
(4) Encystment of Colpoda	(4) Fungicidal effects

Unreported studies in which aerosols of Serratia marcescens were exposed at 40 per cent relative humidity to wave lengths in the region of 2800A to 4100A have illustrated that the air-borne particles below five microns are more susceptible than those larger than five microns (159). This observation is in agreement with that of Bourdillon et al (34) who stated that organisms in small droplet nuclei are more easily inactivated than those in large particles. However, most investigators favor the quantum-hit interpretation because of observed exponential survival curves.

Although as early as 1914 (130) bacterial effectiveness was compared with cellular absorption, biological action spectra using accurately defined monochromatic UV radiation were not published until 1928 (93). A number of authors have since published action spectra for a variety of microbial forms including bacteria (94,185), bacteriophage (85,88,95,327), viruses (153) and nematode eggs (154,164).

Hollaender (149) has shown that nucleic acid absorbs most effectively those wave lengths that also show maximum lethal action against bacteria and fungi (2650A). Absorption curves of typical proteins show peaks of absorption at those wave lengths which are also most damaging to pin worm eggs and to tobacco mosaic virus (below 2500A). Hollaender believed that the action of UV radiation near 2600A functioned through the nucleic acid constituent of the cell and that shorter wave lengths (2250A), because of their lack of penetrability, affected the outside protein layer. Ellis et al (75) have summarized graphically the results of several workers and demonstrated that the wave length of maximum absorption by nucleic acid is in the same general range as those wave lengths reported to give maximum killing of bacteria and paramecia. These data are shown in Figure 2.

Although the exact mode of germicidal action of UV energy is as yet unknown, many of the factors involved in the action have received considerable attention. It has been claimed by Giese (100) that, in contrast to photodynamic reactions, UV radiation acts equally well in the presence or absence of oxygen. The fundamental reaction is thought to be photochemical, and

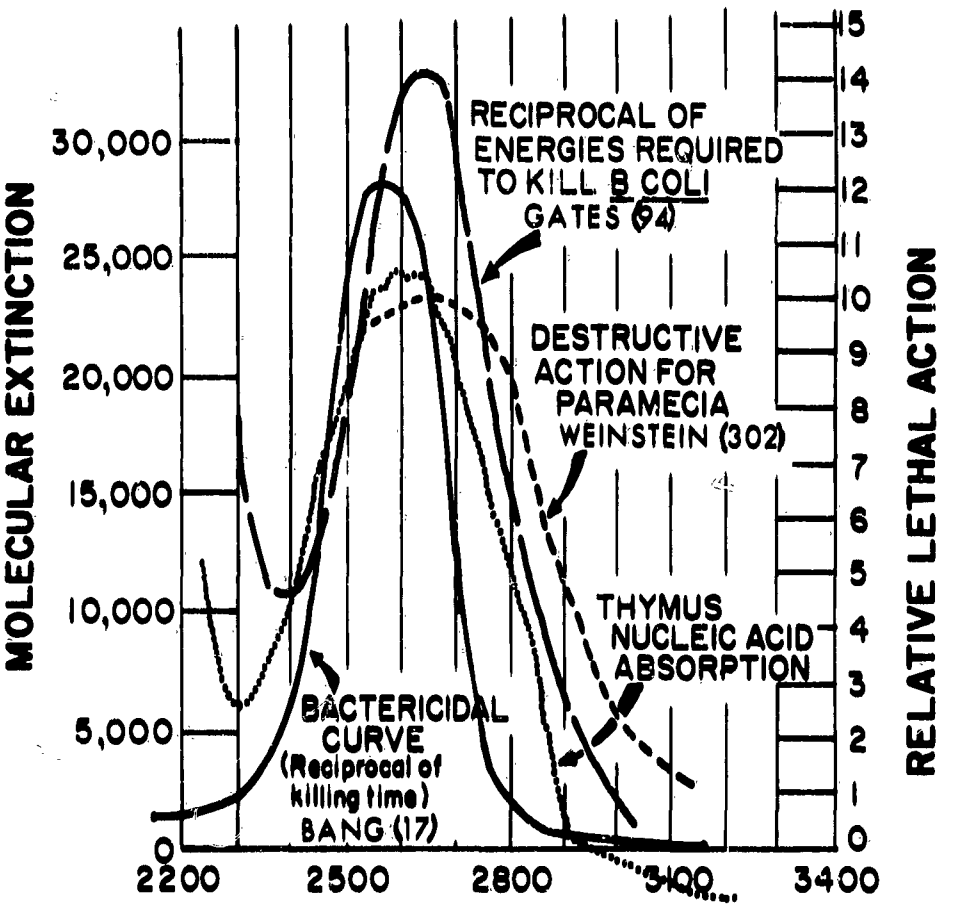

Figure 2. Comparison of Lethal Action of UV and Absorption of UV by Nuclear Materials. Ellis et al (75)

with bacteria the temperature coefficients obtained for the reaction have substantiated this theory (94). For a discussion of the photochemical and cytochemical action of UV radiation, reference is made to Radiation Biology, Vol. II, edited by Hollaender (151).

UV radiation produces its main effect upon the organism absorbing the radiation rather than by affecting the medium. A "single photon hit to kill" theory has been advanced by Lea (183) but with bacteria this has so far been difficult to prove (135,247). According to the Bunsen-Roscoe reciprocity law, the amount of energy required to kill an organism is a product of the radiation intensity and time (I x t = K). For the bactericidal action of UV radiation, this law has been proved to hold true over a range of intensities requiring from only a few microseconds to several hours to produce the same amount of total radiation (247). The law was found not to apply when the time of exposure involved an appreciable part of the life cycle of the organism.

Over a wide range of temperature the resistance of bacteria to UV radiation is not affected qualitatively, providing the temperature used has no deleterious effects on the bacteria in question. UV radiation has been shown to kill fungal spores even at the temperature of liquid air (100).

Several other factors have been recorded by Rentschler et al (247) from experiments in which inoculated agar plates were exposed to UV radiation:

(1) A sublethal exposure of bacterial cells to UV radiation retards the rate at which bacterial colonies will develop.

(2) An individual organism will differ in its resistivity to UV radiation at different stages of its life cycle.

(3) In a given species, different bacteria at the same stage of their life cycle may vary in their resistivity to UV radiation.

C. CHARACTERISTICS OF VARIOUS WAVE LENGTHS

Good agreement has been obtained by different workers concerning the most effective wave length for inactivating various microorganisms. Table II gives the wave lengths found to be most efficient for a variety of organisms, as well as the reference source. The evidence rather conclusively demonstrates that the most effective range for inactivating bacteria and for most virus strains is between 2250A and 2800A.

UV radiation shorter than 2000A is not particularly effective as a germicide. The radiation lacks the penetrating properties of the longer radiation and is absorbed to great extent by air. The germicidal properties of radiations longer than 2800A have been amply investigated. Hollaender (149) has shown that the intensity or exposure time necessary to kill bacteria with energy of longer wave lengths than 3650A is 1,000 to 10,000 times

TABLE II. MOST EFFECTIVE GERMICIDAL WAVE LENGTH

ORGANISM	MOST EFFECTIVE WAVE LENGTH, A	REFERENCE
Bacteriophage	2250	Giese (100)
E. coli bacteriophage	2650	Zelle & Hollaender (327)
E. coli	2650	Hollaender & Claus (152)
E. coli	2652	Wells (305)
E. coli	2650	Gates (94)
E. coli	2537 - 2575	Luckiesh (185)
E. coli	2652	Wyckoff (326)
Influenza A virus	2650	Giese (100)
Paramecium (protozoa)	2650	Weinstein (302)
Rous' sarcoma virus	2300	Giese (100)
Salmonella typhosa	2100 - 2800	Newcomer (223)
S. marcescens	2805	Ehrisman & Noethling (72)
Staph aureus	2537	Gates (94)
Staph aureus	2650	Ehrisman & Noethling (72)
Tobacco mosaic virus	2250	Giese (100)
Tobacco mosaic virus	2250	Hollaender (149)
Vaccinia virus	2650	Giese (100)
Bacteria, unidentified	2800	Cernovodeanu & Henri (44)
Bacteria, unidentified	2750	Mashimo (201)
Bacteria, unidentified	2500	Bang (17)
Bacteria, unidentified	2265 - 3287	Barnard & Morgan (19)
Bacteria, unidentified	2400 - 3020	Bucholz & Von Jeney (38)

that required with radiation shorter than 3000A. Data published by Luckiesh (185) demonstrate that the effectiveness of 2537A is about 4000 times that of 3650A; 10,000 times that of 4047A; 30,000 times that of 5461A; and perhaps 35,000 times that of 5780A.

Studies on the effect of the UV radiation emitted from commercial sunlamps on aerosols of Serratia marcescens at an RH of 40 per cent showed that wave lengths in the range of 2900A to 3450A had approximately 10 times the effect as wave lengths in the 3450A to 7600A range (160).

A great deal of the work on the bactericidal effects of UV radiation was done before the advent of the low-pressure mercury vapor lamp (so-called germicidal lamp). The authors, Weinstein (302), Coblentz and Fulton (46), Hollaender (149), Duggar (70), and Hollaender and Claus (152) used carbon, mercury, and tungsten arc lamps with water-cooled quartz jackets. The radiation emitted from these arcs cover a wide band and must be separated by a system of filters or prisms before quantitative data can be collected.

With the development of the low-pressure mercury vapor lamps, which emit 95 per cent of their UV radiation in the resonance line of 2537A, most of the recent data have been reported on the basis of the quantity of energy in this wave length needed to destroy microorganisms. Wave length 2537A is estimated to possess about 85 per cent the relative germicidal effectiveness of wave length 2650A. However, Buttolph* (151) has suggested that the fact that 2537A is 10 to 20 per cent less effective than the maximum effective wave length is of minor importance because of the experimental errors inherent in determining the optimum bactericidal region. Adoption of a more or less standard ultraviolet source has greatly simplified the problem of correlation of data and has greatly enlarged the practical uses to which UV radiation may be applied.

* Cited in Hollaender.

III. LAMPS WHICH PRODUCE UV RADIATION

A. GENERAL

During the past thirty years approximately 75 new types of UV lamps have been developed and placed on the market. These lamps vary in size, in wattage, and in the emitted spectrum. Many have been designed for specific applications; for example, for photochemical reactions, for sun-tanning and vitamin D production, for light, for bactericidal effect or for ozone production.

Ultraviolet lamps contain mercury vapor. The passage of current through the vapor excites the mercury atoms to various energy states. In making the transition from one state to another the atoms emit radiation of definite wave lengths. The probability of these various transitions depend upon the pressure of the mercury vapor, the amount and type of other gases present, and the electrical conditions in the discharge.

At very low mercury pressures, such as ten microns, most of the emitted radiation is a result of transition from the lowest excited state to the normal state. This is known as resonance radiation. Radiation thus produced may be absorbed by other mercury atoms and re-emitted without change in frequency. In low-pressure, mercury discharge lamps, almost all of the radiation is emitted at wave length 2537A. Increase of pressure results in broadening of the emission lines and in an increase in the continuous background radiation. At very high pressures the radiation approaches that of an incandescent body. In typical quartz lamps the amount of energy emitted below 3800A is greater than the visible energy radiated by a factor of 30 to 50 per cent, depending upon the pressure of the mercury.

Recently, a number of xenon and xenon-mercury arcs have been designed. The envelopes are of fused quartz and the lamps operate at 20 to 40 atmospheres. The lamps are primarily used as light sources, however, a considerable amount of energy is radiated in the UV region.

Mercury in the xenon lamp increases both the visible and the UV radiation. Xenon lamps have been suggested for use as sun lamps.

Sun lamps produce UV radiation such as emitted from the sun. The Council of Physical Therapy of the American Medical Association (7) has proposed that the energy below 2800A should be less than one per cent of the total energy of wave lengths between 2800A and 3132A and that the intensity should be sufficient to produce an erythema on an untanned skin in one hour or less at a distance of 24 inches.

Two general types of sun lamps are manufactured, the mercury vapor type and the fluorescent type. The mercury lamp is located inside a reflector bulb together with a tungsten filament coil which acts as a ballast for the inside lamp. The outer bulb removes all of the radiation below 2800A. These R.S. sun lamps can be operated directly from a normal 110-125V line.

The fluorescent sun lamp is made similar to a regular fluorescent lamp except that the lamp wall is made from a glass permitting most of the radiation above 2800A to escape. A phosphor coating on the inside of the lamp absorbs the 2537A resonance line and produces a fluorescence band having a peak at approximately 3150A. The emission of this lamp closely simulates the UV radiation from the sun. Regular fluorescent ballasts and fixtures are used to operate these lamps.

Application of the sun lamp is mainly for the formation of vitamin D in the skin, to prevent rickets and to produce a "sun-tan". Ronge (258) has found that sun lamps in a schoolroom reduced absenteeism and improved the physical fitness of the children.

The following discussion of UV lamps will be confined primarily to low-pressure, mercury vapor lamps which produce germicidal radiation. Such lamps have pressures of 0.004 to 0.02 millimeter of mercury as compared to high-pressure sources which may operate at 0.5 to 75 atmospheres. Low-pressure lamps were made possible by the development of inexpensive glass capable of transmitting UV radiation. The greatest portion (95 per cent) of their emitted radiation is in the 2537A wave length band (99,312). This wave length is near the most germicidal portion of the spectrum for bactericidal action.

B. GLASS THAT TRANSMITS UV RADIATION

The constituents of ordinary glass, which are the chief absorbents of UV radiation, are iron and titanium oxides. Ferrous ions absorb very little of the UV radiation while ferric ions give high absorption (174). UV transmitting glass shows a decreased radiation transparency after prolonged exposure to high intensities of UV radiation. This phenomenon is called solarization. The transmitting ability is usually improved when the glass is heated. Davidovich (57) in 1930 and Nordberg (226) in 1947 reviewed the status of commercial UV transmitting glass.

The glass commonly used for the manufacture of present day UV tubes has characteristics as follows:

1. Corning Number 9741 Glass

Corning number 9741 glass was originally used as the envelope for all hot and cold cathode UV lamps. This glass had a number of undesirable properties. The glass solarized rapidly and blackened, especially when operated at low temperatures. When new, the lamps sometimes produce excessive amounts of ozone. At the present time number 9741 glass is used only in special 3.5 to 4 watt ozone producing bulbs.

2. Corning Number 9823 Glass

This is the glass now used in the manufacture of all hot cathode lamps. The glass does not solarize or blacken as rapidly as Corning 9741 glass. It does not transmit radiation below 2000A and therefore does not produce ozone.

3. Corning Vycor Number 7910 Glass

This glass is made by leaching the flux from alkali-borosilicate glass and consolidating by heat the high silica residue (211). The glass is used in the manufacture of cold cathode and Slimline type UV tubes. This glass is in some respects similar to quartz (96% silica and 4% alumina), therefore, UV transmission properties are similar. The transmission of 2537A radiation is about 80 per cent, the rate of solarization is negligible, and very little darkening occurs even when operated at low temperatures. This glass transmits less than 0.1 per cent of radiation below 2000A which produces only a negligible amount of ozone.

4. Corning Vycor Number 7912 Glass

This glass is used in the manufacture of some cold cathode and Slimline lamps. It has the same characteristics as the 7910 glass except that a controlled amount, approximately 2.0 per cent, of short ozone-producing radiation is transmitted. Lamps from this glass are called "high-ozone lamps." Older quartz UV lamps transmitted from 50 to 90 per cent of radiation below 2200A. At the present time some quartz lamps use a Vycor or quartz jacket with an acetic acid solution to filter out radiation below 2200A.

The maintenance of UV output by these glasses is shown in Figure 3. There are three types of low pressure mercury vapor lamps and one high-pressure lamp used for bactericidal purposes.

C. HOT CATHODE GERMICIDAL LAMPS

The operation of these lamps is similar to standard fluorescent lamps. They operate at a low voltage from a ballast or transformer and for starting require a device such as a glow-switch to preheat the electrodes. Argon gas is used to facilitate starting. The electrodes are located in the ends of the lamp and consist of tungsten filaments coated with emission material (calcium, barium, and strontium oxides). The life of the lamp is governed by the life of the electrodes and the rate of solarization of the glass. If the lamp is turned on and off frequently its life will be shortened. Corning number 9823 glass is now used for the fabrication of the glass tube.

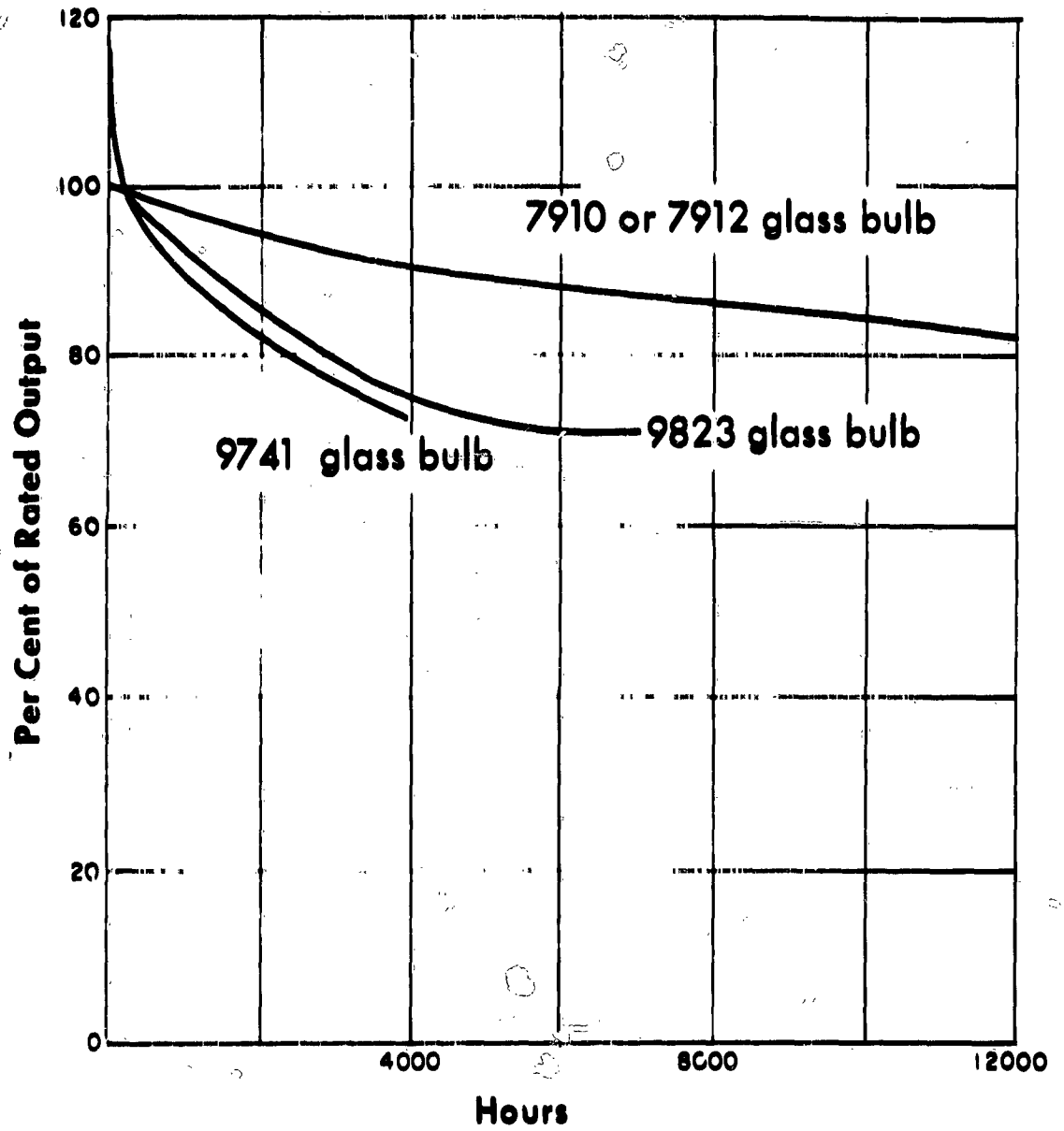

Figure 3. UV Output of Bactericidal Lamps Made from Various Glasses. Westinghouse Electric Corporation (313)

One of the important characteristics of hot cathode lamps is the effect of tube wall temperature on the UV radiation output. Operation at low temperatures results in lamp blackening and low output. Also, starting the lamp at low temperatures is sometimes unreliable and may require special equipment. Figure 4 shows how the tube wall temperature affects UV radiation output. When operating with a tube wall temperature of 32°F, the output is less than one tenth that of the output obtained when the wall temperature is 100°F. The most efficient ambient temperature for operation is around 80°F. Operation in strong air currents also shortens the life of this lamp and lowers the output of UV radiation.

D. COLD CATHODE GERMICIDAL LAMPS

This lamp, containing argon and neon gas, is equipped with cylindrical nickel electrodes and is made from Corning Vycor number 7910 or 7912 glass. The lamp requires a transformer to obtain high voltages for starting and operating, but no preheating of the electrodes is required. Thorium metal inside of the electrode increases the electron emission. The life of the lamp is governed mainly by the ability of the glass tube to transmit UV radiation. The electrodes operate "cold" and seldom wear out. The lamps may be operated at refrigerator temperatures without excessive lamp blackening, however with some loss of UV radiation output. Instantaneous starting is obtained at low temperatures.

The relative UV radiation output per rated lamp watt from the cold cathode lamp is of the same order of magnitude as obtained from the hot cathode type. Two types of cold cathode lamps are commercially available; one is designed for high ozone production and the other for low ozone production. The difference is the glass used in the lamps.

E. HIGH-INTENSITY GERMICIDAL LAMPS (SLIMLINE)

This lamp has characteristics in common with both the hot and cold cathode lamps. It utilizes a high starting voltage which gives instant starting. Although the electrodes are cold when the lamp is started, after the start it operates with the electrodes hot. The life of the lamp, as in the hot cathode type, depends mainly on the life of the electrode which in turn depends on the frequency of starts.

The outstanding feature of the Slimline lamp is its high output. The lamps may be operated at four different currents (120, 200, 300, and 420 milliamperes). The UV radiation output per rated lamp watt is higher than that obtained with the hot cathode lamps because of the higher transmission of the glass. The higher intensities obtained make it useful for installations requiring large amounts of 2537A radiation. The ozone output is rated as negligible. This lamp is also available in Corning Vycor number 7912 glass which emits some 1849A radiations and thus produces small amounts of ozone.

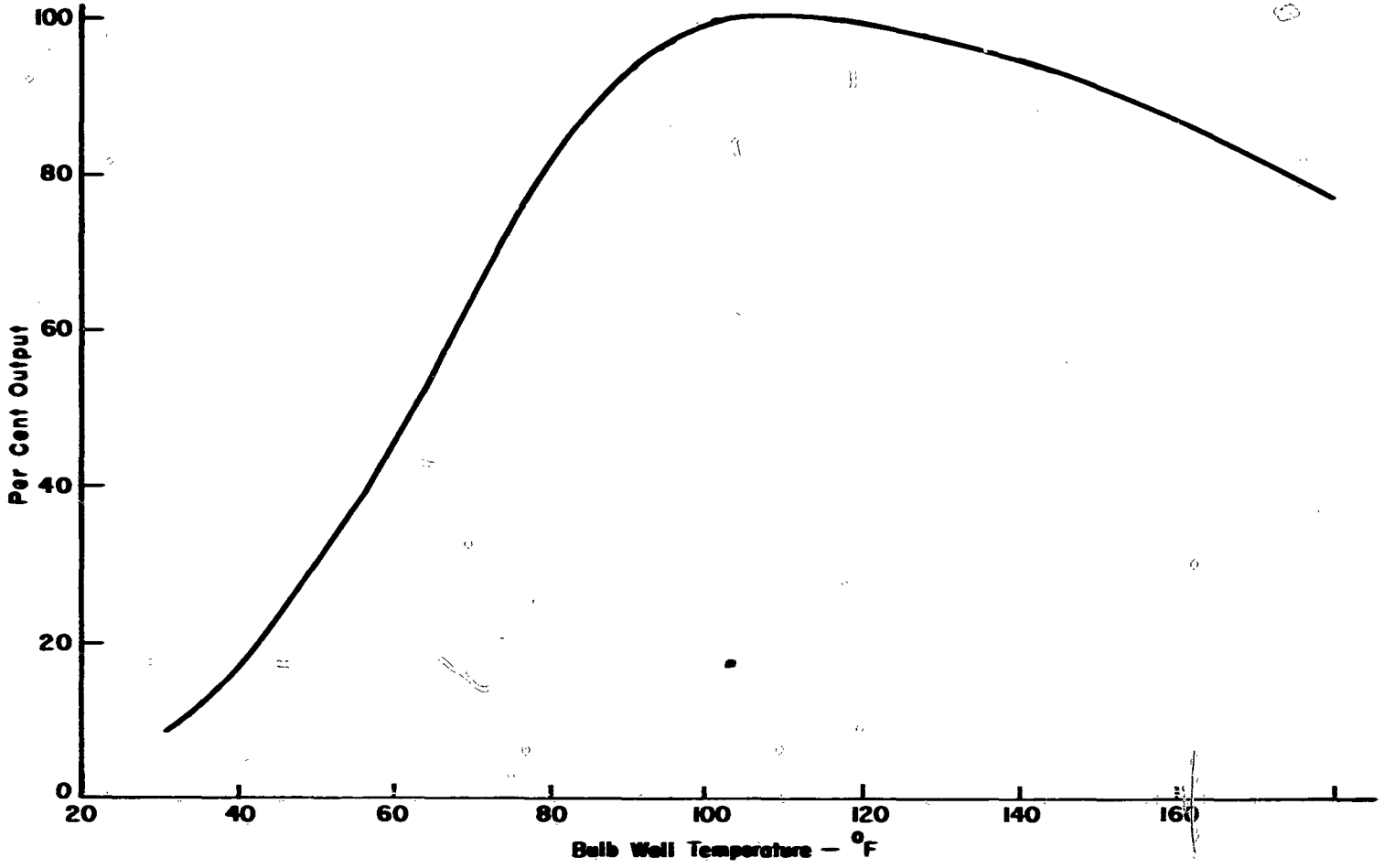

Figure 4. Effect of Bulb Wall Temperature on the UV Output of Low Pressure Mercury Vapor Lamps. General Electric Company (99)

Table III shows some of the characteristics of the three types of lamps discussed above.

TABLE III. CHARACTERISTICS OF THREE TYPES OF UV LAMPS

	HOT CATHODE	COLD CATHODE	HIGH-INTENSITY
Rated watts	30	17	39
Over-all length	36"	34-3/4"	36"
Tube diameter	1"	5/8"	6/8"
Operating voltage	103-108	410	130
Operating current in amps	.34	.050	.420
Related life hours	7500	17,500	7500
UV intensity at 1 meter in microwatts per sq cm	72-80	46	120
UV output in watts of 2537A	7.2-8.4	5.2	13.8

The figures listed by lamp manufacturing concerns for UV lamp output are average values obtained from readings on many lamps (133,220). The output of individual UV lamps is known to vary more than the visible light output of lighting lamps.

F. HIGH-PRESSURE MERCURY LAMPS

For routine practical use, high-pressure lamps offer few advantages and are useful only for special research purposes. The following summarizes briefly their essential characteristics:

(a) The efficiency of the conversion of input electrical energy to output radiant energy is less than with low-pressure lamps. However, the germicidal output per unit of high-pressure quartz lamps is generally greater.

(b) The energy emission is distributed over a wide range of wave lengths (about 20), therefore, much of the UV is not in the spectral area of maximum germicidal effectiveness.

(c) The rated life is generally shorter than with low-pressure lamps.

(d) As a high-pressure lamp solarizes and depreciates, the intensity of different wave lengths may decrease at variable rates.

(e) The transformer necessary to operate a high-pressure mercury lamp is generally large and expensive.

(f) High-pressure mercury lamps made of quartz produce a great deal of ozone unless enclosed in a special ultraviolet transmitting jacket.

Further information on types of high-pressure UV lamps may be found in publications by Ellinger (74) and Meyer and Seitz (210).

G. EXPLOSION-PROOF LAMP FIXTURES

Several kinds of explosion-proof UV lamp fixtures are manufactured. These are actually lamp fixtures equipped with explosion-resistant Vycor or quartz glass housings. Regular UV lamps are placed inside the housing. The fixtures may be used in areas which are considered hazardous because of the presence of flammable vapors, gases, or combustible dusts. In addition to the high initial cost of these fixtures (approximately $125.00 each) the UV radiation output of the lamps is somewhat lowered because of absorption by the Vycor glass housing. Explosion-proof lamp fixtures are manufactured by Hanovia Chemical Manufacturing Company and by Crouse-Hinds Manufacturing Company.

Conversion of the Westinghouse type SB-30 fixture, for cold cathode lamps, to produce a water-tight unit (not explosion-proof) can be done at a cost of about $52.00 per fixture, including the price of the fixture and lamp. Such a design has been accomplished on a local basis, and tests have shown such a unit safe to operate in areas of high humidity or where rooms are washed with liquid solutions. The converted fixture is shown in Figure 5. The transformer has been placed in an aluminum box with a gasket lid. Neoprene membranes are used to produce water-tight seals at the socket connections at each end of the UV tube. This fixture satisfactorily resisted sprays of brine solution without shorting or gathering moisture on critical parts and without significant change in UV radiation output.

H. UV RADIATION FROM WHITE LIGHT SOURCES

1. Incandescent Lamps

Most artificial sources of white light are weak sources of UV radiation. Photoflood lamps are probably the highest generators of incidental UV radiation. The glass walls of ordinary incandescent type lamps will not permit the passage of radiations shorter than 3200A. Koller (174) presented data showing the relative amount of energy in various wave length bands produced by several types of incandescent lamps (Table IV). All five lamps

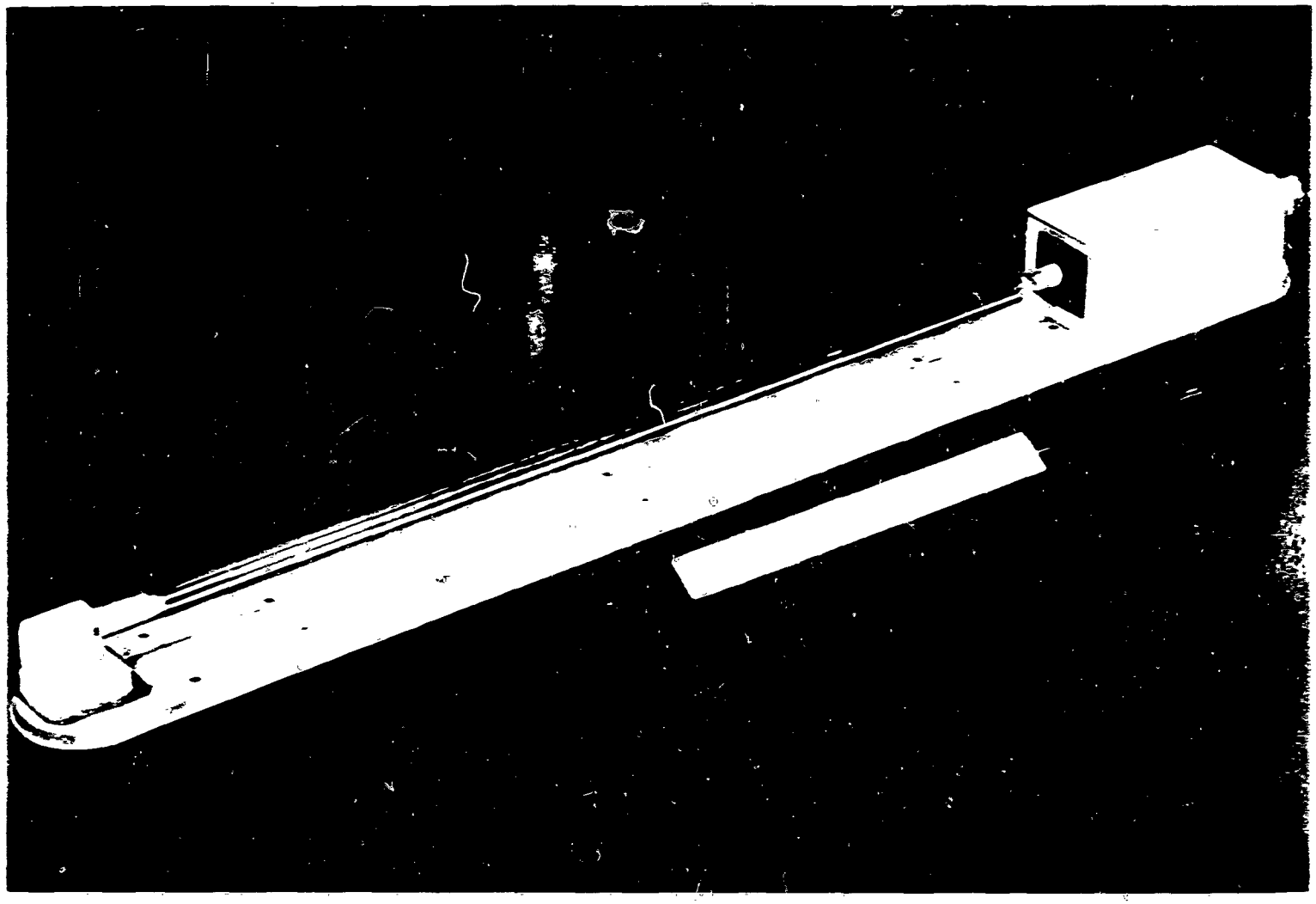

Figure 5. Moisture-proof UV Fixture. (EF Neg C-3230)

listed produced UV radiation in the 3200A to 3800A wave length band. Three of them produce detectable amounts in the 2800A to 3200A band. According to Koller, total UV radiation of less than 3800A emitted by the 1000-watt photoflood lamp (life of 10 hours) is approximately three watts.

TABLE IV. RADIATION FROM INCANDESCENT LAMPS

Energy in various wave length bands falling on a unit area at a distance of 1 meter from the lamp. (Koller 174)

TYPE OF LAMP	MICROWATTS PER SQUARE CENTIMETER		
	2800A-3200A	3200A-3800A	3800A-7600A
40 watt standard		0.08	22.5
100 watt standard		0.45	75
500 watt standard	0.045	3.5	440
1000 watt standard	0.28	8.8	1160
1000 watt photoflood	1.4	25	1580

2. Fluorescent Lamps

Fluorescent lamps also emit small amounts of UV radiation. In fact, in its simplest form, the fluorescent lamp is an UV lamp which utilizes a glass envelope coated on the inside with special fluorescent powders or phosphors. About 60 per cent of the wattage input of a fluorescent lamp is used to produce radiation concentrated at 2537A wave length. This radiation is produced inside the lamp and is used to excite the phosphors which produce the white light. The over-all efficiency of this type of lamp in the production of light is about 10 per cent.

Although the amount is very small, some UV radiation is emitted by fluorescent lamps, the amount varying with the type of phosphor material used. The data shown in Table V are taken from a table by Luckiesh and Taylor (187) and show the intensity of UV radiation shorter than 3150A produced by sunlight and by a fluorescent light. The foot-candle concentration of the white light is given for each source. Although direct sunlight at 6600 foot-candles contains 70 times as much UV radiation as fluorescent light at 50 foot-candles, it is obvious that higher intensities of fluorescent light will be proportionally greater. For instance, another way to express the relationship shown by Luckiesh and Taylor would be to calculate the microwatts per square centimeter of UV radiation produced per foot-candle of light. Fluorescent light produces 0.016 microwatts per square centimeter of UV radiation shorter than 3150A for each foot-candle of light

by this method, while the same figure for direct sunlight is 0.0085. On this basis the UV output of fluorescent lamps per foot-candle appears to be less than skylight with a clear blue sky, or sunlight and skylight combined, but more than direct sunlight.

TABLE V. UV FROM FLUORESCENT LAMPS
(Luckiesh & Taylor 187)

Intensity of UV radiation shorter than 3150A

	FOOT-CANDLES	MICROWATTS PER SQUARE CENTIMETER	
		Absolute	Relative
Direct sunlight	6600	56	70
Skylight-clear blue sky	1900	117	146
Sunlight and skylight	8500	173	216
Direct fluorescent light 3500° white	50	0.8	1

There is no doubt that the germicidal effectiveness of the longer UV wave lengths discussed above are of a very low order, but the fact that these weak sources of UV radiation exist is worthy of consideration. It is obvious that unless all culture work is done entirely in the dark or unless special visible light sources are used, small amounts of UV radiation from the usual lighting system are apt to be present in all biological laboratories.

3. Survival of Bacterial Spores on Fluorescent Lamps

Two 15-watt daylight fluorescent lamps, mounted in receptacles, were placed in a ventilated cabinet. One lamp surface was contaminated with a cotton swab soaked in a suspension of Bacillus subtilis var. niger spores containing 2×10^9 spores per milliliter. The other lamp was treated similarly with a 2×10^7 spore suspension.

Both lamps were turned on after contamination was completed and "zero" time surface samples taken. A portion of the surface of each lamp was sampled at intervals of 30 minutes for a period of 11½ hours and at the end of 24 hours.

The control experiment was conducted in the same fashion with the exception that lamps remained off. On the unlit lamp contaminated with the 2 x 10^9 spores suspension, surface samples remained TNTC for 9 days; on the 2 x 10^7 lamp, samples were TNTC for 8 days, and 226 colonies were recovered at 8 days. Sampling was discontinued at 9 days.

The results of the tests with burning lamps are shown in Table VI. Since the temperature at the surface of a fluorescent lamp is not sufficient to cause inactivation of bacterial spores, it is likely that the results obtained were due, in part, to the action of ultraviolet radiation on the spores. If one assumes that approximately 24 hours is required for the lethal action and that a dose of 800 microwatt minutes of 2537A is necessary to inactivate a spore population (see page 59), it can be theorized that the total radiation at the surface of the lamp had a germicidal equivalent of 1.8 microwatts per square centimeter of 2537A.

TABLE VI. RECOVERY OF BACILLUS SUBTILIS SPORES FROM BURNING FLUORESCENT LAMPS

SWAB SITE	CONTROL*	TIME OF SAMPLING							
		30 min	1 hr	6 hr	6.5 hr	7 hr	11 hr	11.5 hr	24 hr
Bulb 1	0	TNTC	TNTC	TNTC[d]	TNTC[d]	TNTC[d]	85	15	13
Bulb 2	0	TNTC[d]	TNTC[d]	100	72	35	0	1	1

Bulb 1 - Contaminated from 2 x 10^9 spore suspension.
Bulb 2 - Contaminated from 2 x 10^7 spore suspension.
TNTC - Growth confluent; no distinct colonies.
TNTC[d] - Colonies distinct but TNTC.
* Surface sample of lamp prior to contamination.

IV. REFLECTANCE OF UV RADIATION

A. LITERATURE REVIEW

All radiant energy is reflected to some extent from surfaces. The amount reflected depends upon the type of surface, the wave length of the energy, and the angle of incidence. Luckiesh and Taylor (188) reported experiments to determine the percentage of UV energy (2537A) reflected by various surfaces. A specially designed reflectometer was used with a Bausch and Lomb number 2800 quartz monochromator for spectral measurements. The authors concluded that aluminum is by far the best reflecting medium for 2537A UV radiation. Table VII, prepared from data presented by Luckiesh and Taylor, shows the per cent reflectance of 2537A energy from the various surfaces tested.

TABLE VII. REFLECTANCE OF UV RADIATION FROM VARIOUS SURFACES
(Luckiesh & Taylor 188)

MATERIAL	PER CENT REFLECTANCE OF 2537A
Aluminum metal, etched and brightened	88
Aluminum metal, bright rolled	84
Aluminum metal, foil	73
Aluminum metal, Alzak treated	65 - 75
Aluminum metal, mill	40 - 60
Aluminum paint	40 - 75
White-coat plaster	40 - 60
Chromium metal	45 - 55
Stainless steel	20 - 30
Wall paper (ivory and white)	21 - 31
Acoustic plaster and wallboard	10 - 20
Vitreous enamel	5 - 10
Kalsomine white water paint	12
Alabastine white water paint	10
Average oil paints	5 - 10
White porcelain enamel	4.7

In another series of tests (185) in which nine different aluminum paints were used, it was found that when painted surfaces were exposed to high intensity 2537A energy the reflectance for longer wave lengths was increased 12 per cent. Reflection coefficients ranging from 48 to 66 per cent were obtained for the nine brands of aluminum paint. Several important points regarding reflectance of UV radiation are:

(a) Aluminum metals and aluminum paints are the best reflectors of UV energy. Intensive and prolonged exposure of aluminum paint does not seem to destroy its ability to reflect the radiation. Such a paint, however, should be made of pure aluminum flakes, and the plastic lacquer should have high UV transmission properties and stability.

(b) Stainless steel has approximately 50 per cent the reflectance efficiency of aluminum. For maximum reflectance, reflectors made with stainless steel or any other metal except aluminum should be painted with aluminum paint.

(c) Oil paints and some water-soluble paints are poor reflectors of UV radiation. Reflectance depends upon the type of pigment in the paint; zinc oxide pigment usually gives low reflectivity. Oil paints usually give from five to ten per cent reflectance and water-soluble paint from 10 to 12 per cent reflectance.

(d) White wall plaster has reflectance values on the order of 40 to 60 per cent of 2537A.

Alsak aluminum metal gives high reflectance values and is suitable for use as reflectors for UV lamps (Figure 6). Alsak is a trade-mark registered by the Aluminum Company of America. The aluminum is first brightened by an electrolytic method to remove surface impurities and then oxidized to provide a thin coating of aluminum oxide to prevent weathering.

The average reflection coefficient of human skin for 2600A radiation is four per cent (189). There is little difference between tanned and untanned skin.

The amount of radiant energy reflected from the surface of any transparent medium, such as glass, will vary with the angle of incidence. At normal incidence (10 to 60 degrees) the reflection from glass of 3082A UV radiation is about five per cent. Per cent reflectance rises rapidly at angles greater than 75 degrees.

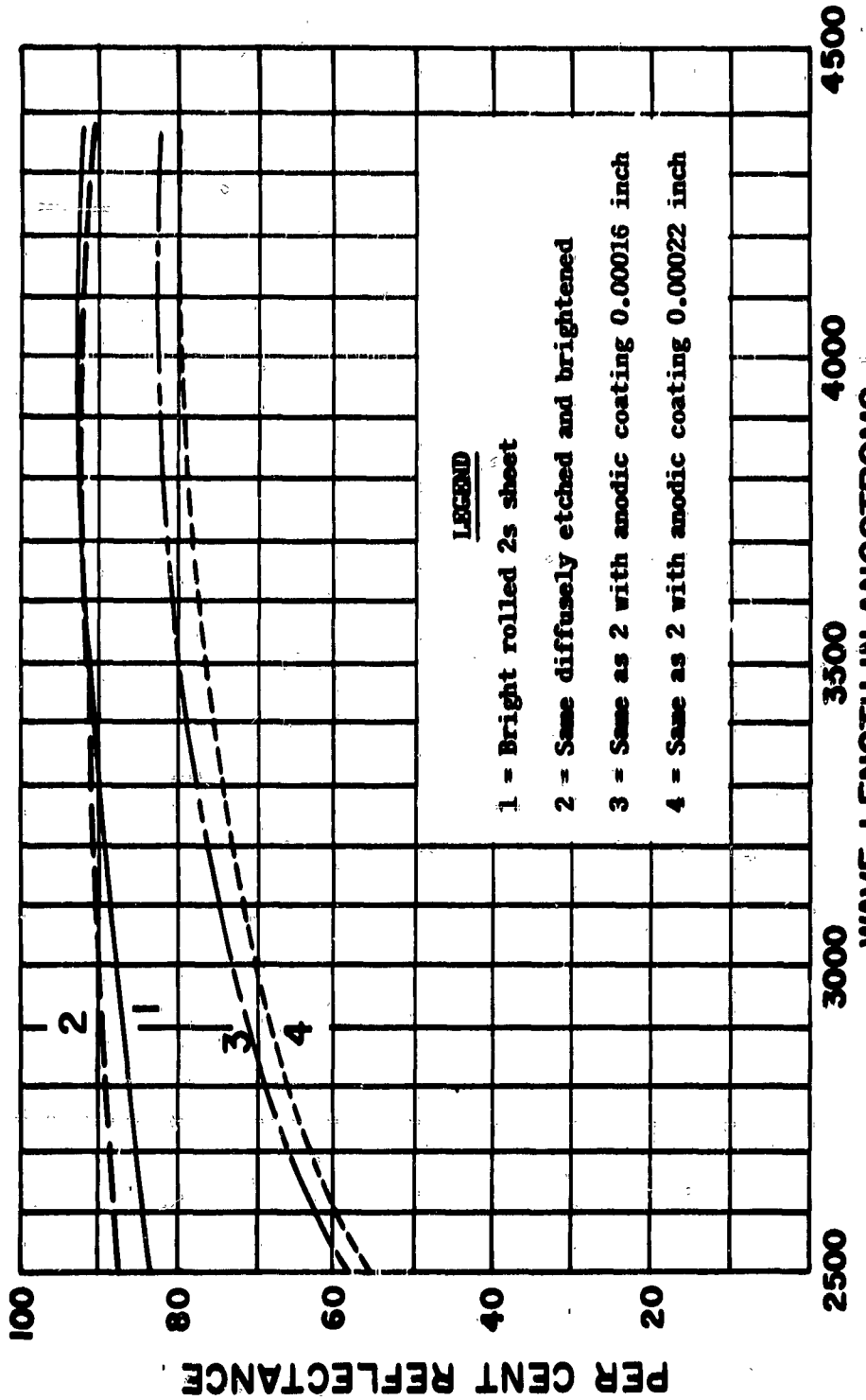

Figure 6. Spectral Reflectance of Alurinum With and Without Alzak Treatment. Koller (174)

Koller (174) gives the following reflectance values for several surfaces:

Reflection of 3000A UV radiation from the ground

Surface	Per Cent Reflection
Fresh snow	85
Dry dune sand	17
Sandy turf	2.5
Water	5

Anderson (10) gives the following reflectivity values for 2537A radiation:

Surface	Per Cent Reflection
Glass (brick, mirrors)	4 - 6
Enamels (baked, any color and white)	5 - 10
Oil paints (lead, any color and white)	5 - 10
Oil paints (zinc, any color and white)	3 - 6

Anderson also reports that water paints and paper frequently have high reflectivity values. This would be true especially for very large angles of incidence.

In the use of UV radiation for control of infectious hazards, aluminum paint or metal should be used if a high degree of reflectance is desired, and oil or suitable water paints should be used if the reflectance is to be minimized.

In connection with the use of reflectors, Buttolph* (151) has pointed out that the mercury vapor in UV lamps will completely absorb 2537A radiations entering the tube from the outside. The 2537A radiation generated in the center of the tube is also absorbed by the mercury vapor and re-radiated to the atoms close to the wall of the tube until it finally escapes. Therefore, to obtain a high intensity of radiation in one direction, the bare lamps should not be placed side by side. Placement of parallel lamps individually in front of reflectors on centers three or four times the lamp diameter was recommended by Buttolph.

B. EXPERIMENTAL

1. Increase in Intensity of Radiation by Use of Reflectors

Tests were made to determine what practical increase in intensity of UV radiation might be expected by the use of reflectors. Measurements were taken before and after the installation of aluminum reflectors in a door barrier installation.

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* Cited in Hollaender.

A considerable increase in intensities was obtained after the installation. Table VIII shows a typical set of measurements for comparison. An increase of over 100 per cent in UV intensities was obtained by the use of five reflectors. It is evident that in some applications the use of aluminum reflectors will permit more efficient and economical utilization of the germicidal energy. The cost of the reflectors used in this installation was approximately $2.00 each. The use of a properly designed parabolic aluminum reflector behind an UV lamp will direct the radiations of the entire lamp in one direction, and by this means intensities in this direction can be increased three- to five-fold.

TABLE VIII. INCREASE IN UV INTENSITIES THROUGH THE USE OF ALUMINUM REFLECTORS

	READING BEFORE INSTALLATION OF REFLECTORS	READING AFTER INSTALLATION OF REFLECTORS
	66	132
	52	108
	36	92
	28	72
	30	75
Totals	212	479

2. Use of Reflectors with Cold Cathode Lamps

Studies had previously been made of air locks and door barriers in installations utilising hot cathode lamps without reflectors. A series of UV intensity measurements were made in an UV door barrier using hot cathode lamps with reflectors, cold cathode lamps without reflectors, and cold cathode lamps with reflectors. The object was to determine if suitable UV intensities could be obtained when cold cathode fixtures (17-watt) were substituted for the hot cathode fixtures (30-watt). Specular Alzak aluminum reflectors were used. Examination of the data obtained showed the following results:

(a) When hot cathode lamps are used with reflectors, the intensities obtained far exceed the minimum required for efficient operation of door barriers.

(b) When five cold cathode lamps without reflectors are used in place of the five hot cathode lamps, the intensities obtained were inadequate for the purposes for which the barrier is intended.

(c) When five cold cathode lamps with aluminum reflectors were substituted for the hot cathode lamps, the intensities were adequate.

As a result of these tests, the standard UV door barrier was designed to utilize five 17-watt, cold cathode UV lamps, each equipped with a properly designed Alzak aluminum reflector.

It was also evident from these tests that the cold cathode lamp would be suitable for use in air locks and other installations. Higher radiation intensities can be obtained if needed by the use of Slimline lamps.

3. Aluminum Paints

The reflectance and resistance to UV radiation of two commercial brands of aluminum paint were investigated. Glidden brand paint* was compared to Rust-Oleum #470.** The reflectance test using 2537A UV radiation showed that the reflecting abilities of metal surfaces painted with both paints were practically the same. Glidden paint reflected about three per cent more than did the Rust-Oleum paint (about 50 per cent reflectance). No deterioration of either surface was evident after exposure of both painted surfaces to UV radiation. The total radiation dose would be equivalent to that received in two years in an UV air lock with intensities of 20 to 30 microwatts per square centimeter. Another sample of Glidden paint was exposed for a period of eight months to an UV radiation intensity of 1500 microwatts per square centimeter without any indication of deterioration.

The effect of aluminum paint in increasing intensity levels by providing better reflecting surfaces is illustrated by tests done on an animal cage rack.

The rack had solid metal shelves located 17 inches apart. UV lamps were mounted horizontally at each end of each shelf. The area between shelves received the benefit of any reflected radiation from the bottom of the shelf above. Using calibrated lamps and meters, intensity measurements were made at various locations between the shelves when the reflecting surface of the cage rack shelf above was painted, first with aluminum paint, and then with a semi-gloss, oil base paint. Typical readings are shown below in milliwatts per square foot:

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* The Glidden Company, Cleveland 2, Ohio.
** Rust-Oleum #470, Ready Mixed, Aluminum - LO, Rust-Oleum Paint Company.

UV RADIATION REFLECTED FROM A SURFACE

Painted Aluminum	Painted Semi-Gloss
30	5
15	4
10	3
4.5	1.5

4. Reflector Design

Experiments to determine proper reflector shapes for UV lamp fixtures have been conducted. It was desired in these tests to design polished aluminum reflectors capable of producing narrow parallel beams of high intensity radiation such as would be required on UV cage racks and in UV door barriers.

Parabolic shapes are necessary to concentrate radiations from UV lamps. To obtain a narrow emission band, a parabola with as large a focal length as is practical should be employed. The center of the UV tube should be placed at or slightly in front of the focal point for maximum efficiency.

For convenience small, cold cathode lamps and fixtures were used. Intensity and radiation patterns were appraised by plotting spatial distribution curves on polar coordinate graft paper. Spectral planes horizontal and vertical to the lamps and fixtures were plotted.

Parabolic polished aluminum reflectors for testing were constructed as shown in Figure 7 with a focal length of 3/8 inch. For comparison, reflectors of an irregular shape intended for use on UV cage racks were also tested. Spatial distribution curves were plotted of the bare lamp, the lamp with the irregular reflector, and the lamp with the experimental parabolic reflector. Results obtained in the vertical and horizontal plane are shown in Figure 8. The experimental parabolic reflector produced a wide flat beam of radiations on a flux intensity in the beam at least five times greater than that of the bare lamps and twice that of the lamp with the irregular reflector.

Based on these experiments, it was recommended that parabolic shape reflectors be used on all UV fixtures where it is desired to project a narrow beam of high intensity radiation. For hot cathode lamps (one-inch diameter) the parabola should have a focal length of 5/8 inch, cold cathode and Slimline lamps, a 3/8-inch focal length parabola.

5. Distribution of Radiation from a Cold Cathode Fixture

In some applications of UV radiation, such as in air locks, it is desirable to have a uniform distribution of energy rather than a concentrated beam. Generally the cold cathode lamp in a Westinghouse SB fixture is used. The portion of the fixture under the lamp has a small V shaped

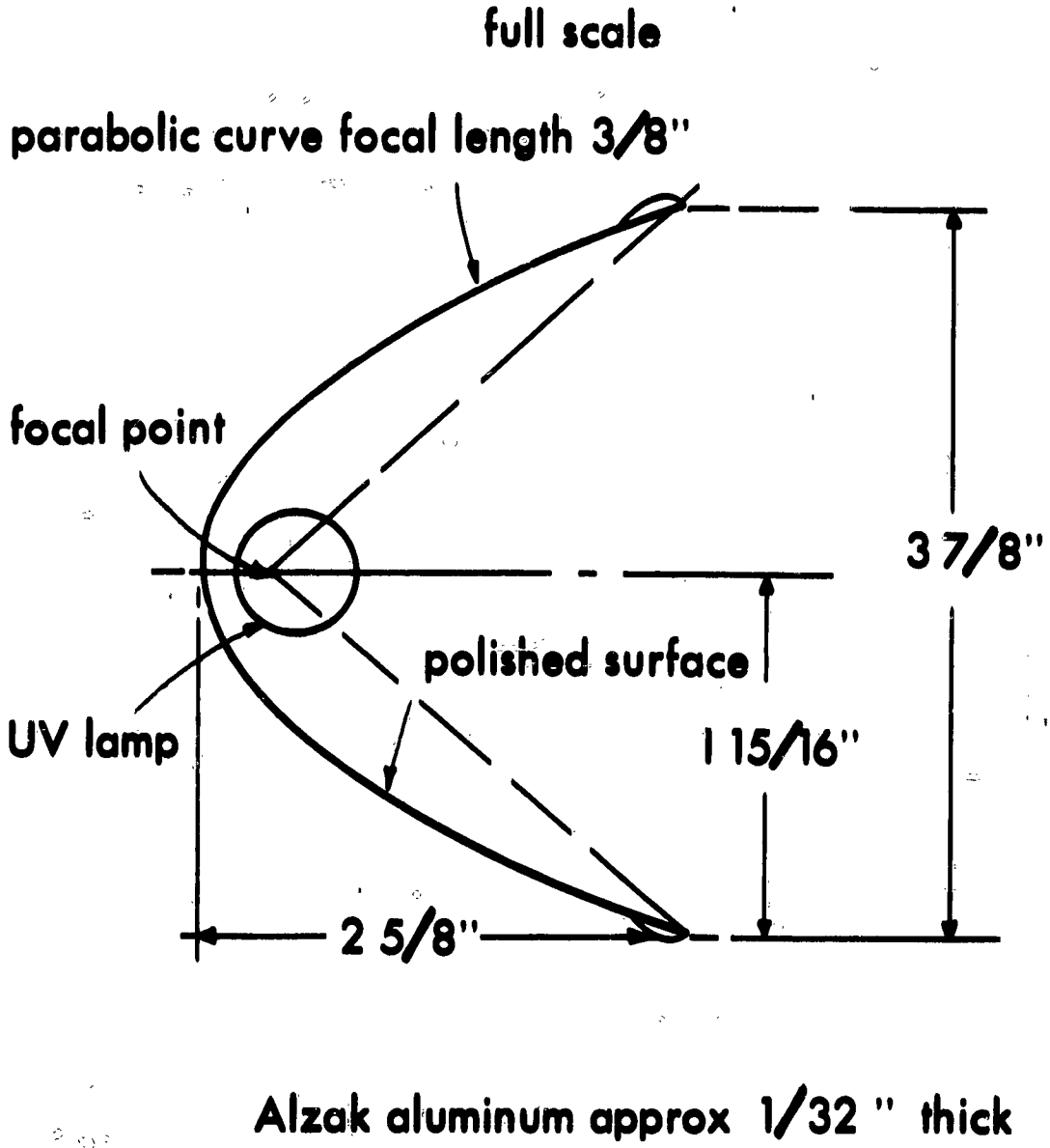


Figure 7. Parabolic UV Reflector.

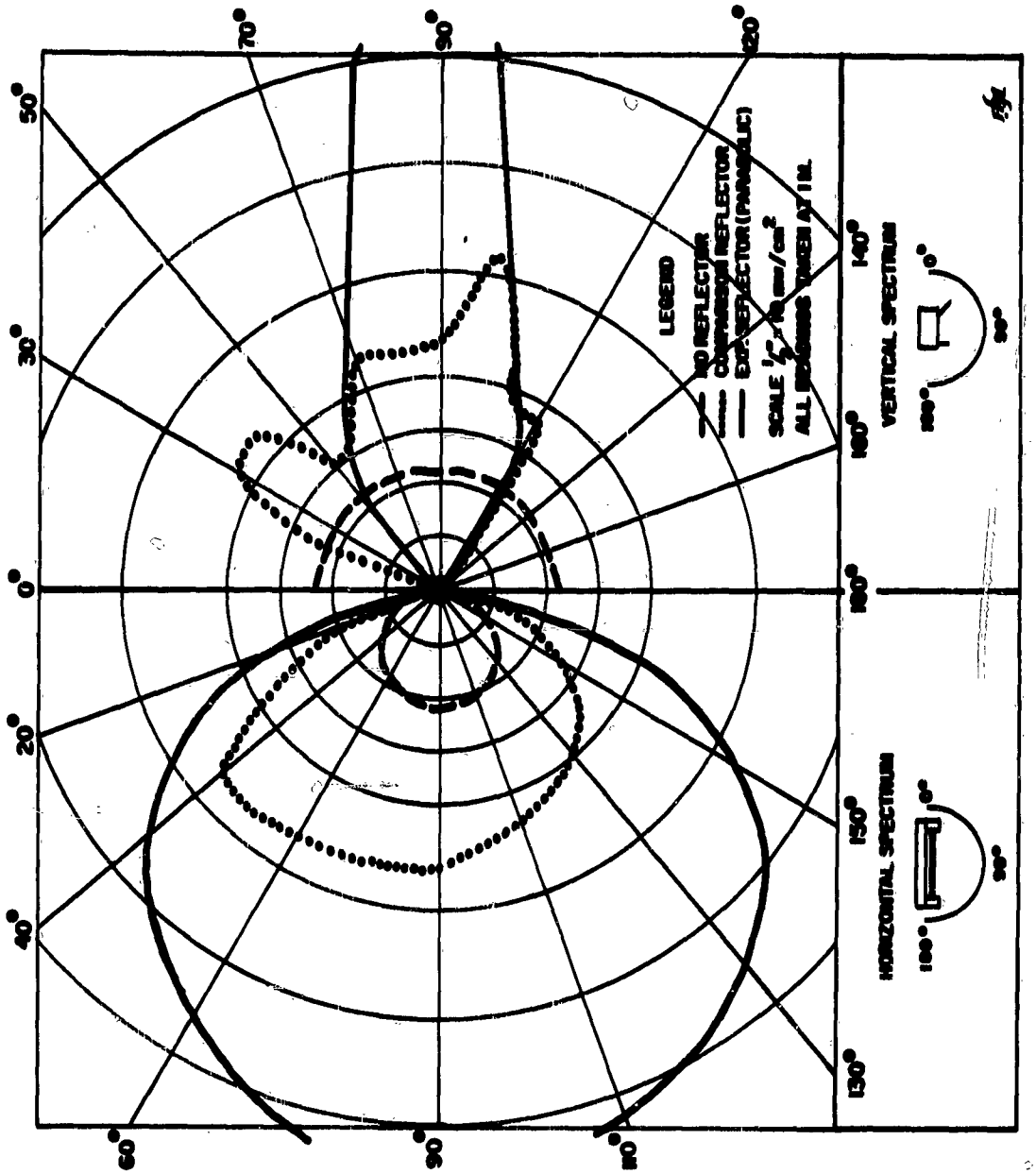


Figure 8. Distribution Curves for Two Reflector Designs.

piece of aluminum which acts to reflect radiation. The spectral distribution is given in Figure 9. The UV output in microwatts per square centimeter at various distances is given in Table IX.

TABLE IX. UV INTENSITIES AT VARIOUS DISTANCES FROM A 17-WATT COLD CATHODE LAMP IN A SB-30 FIXTURE

VERTICAL DISTANCE FROM LAMP, feet	MICROWATTS PER SQUARE CENTIMETER				
6	17.8	17.4	17.0	15.3	13.0
7	13.3	13.3	13.2	12.2	11.2
8	10.7	10.7	10.6	10.4	9.4
9	8.9	8.9	8.8	8.4	7.9
10	7.0	7.1	7.0	6.9	6.6
11	5.9	5.9	6.0	6.0	5.7
12	5.0	5.1	5.1	5.2	5.3
13	4.4	4.5	4.5	4.4	4.4
HORIZONTAL DISTANCE FROM LAMP, feet	0	2	3	4	5

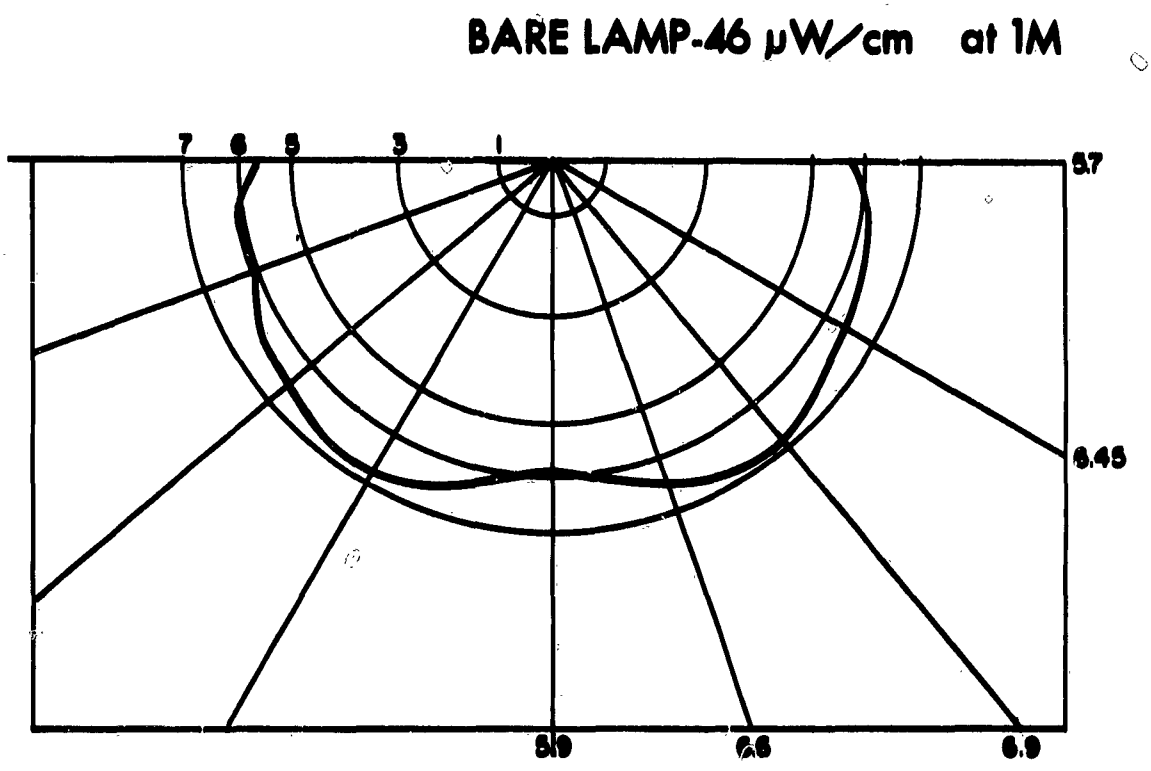


Figure 9. Distribution from a Cold Cathode Lamp (782-30) Installed in a SB-30 Fixture.

V. TRANSMISSION OF UV RADIATION

A. LITERATURE REVIEW

The extent to which various materials permit the passage of UV radiation has been reported at length in the literature, although the exact wave lengths used have not always been accurately defined. It is important to designate the portion of the spectrum being used when interpreting such experimental data. Ellis, et al (75) refer to work done by Moss and Knapp where the investigators found that four millimeter sheets of Pyrex glass transmitted 40 per cent of the erythemal UV radiation used. The erythemal range includes those wave lengths between 2800A and 3200A. Luckiesh and Taylor (188) demonstrated that Pyrex glass will not transmit the shorter wave lengths of 2537A. The relative amounts of energy of various wave lengths emitted by low-pressure mercury discharge lamps is shown in Table X.

TABLE X. RELATIVE AMOUNTS OF ENERGY OF VARIOUS WAVE LENGTHS EMITTED BY TYPICAL GERMICIDAL LAMPS
(General Electric Co. 99)

WAVE LENGTH, A	RELATIVE ENERGY, A
2537	95.15
2652	.16
2804	.04
2894	.10
2967	.43
3022	.22
3130	1.90
3650	2.00
1850 trace - according to type of lamp	

Luckiesh (185) investigated the extent to which 2537A radiations penetrated various types of glass. The data shown in Table XI are taken from this work and show the transmission abilities of 18 different kinds of glass. Table XII shows the transmission of several wave lengths through four types of Pyrex glass. Estimation of the relative bactericidal effectiveness of two wave lengths is included. Other data are shown in Figure 10.

TABLE XI. TRANSMISSION OF 2537A UV RADIATION BY DIFFERENT TYPES OF GLASS (Luckiesh 185)

TYPE OF GLASS	THICKNESS mm	PER CENT TRANSMISSION
Glass Found to Transmit 2537A		
Corning No. 9740*	1	83
Corning No. 9741*	1	66
Corning No. 9720	1	45
Corning No. 9700	1	7
Corning Corex D	1	1
Glass Found Not to Transmit 2537A		
Corning No. 774 (Chemical Pyrex)	1	
Lead glass	1	
Lime glass	1	
Corning No. 772 (Nonex)	1	
Miscellaneous Pyrex	1.05	
Microscope glass	0.18	
Microscope slide	0.96	
Quartzlite	2.02	
Helioglass	2.00	
Vitaglass	2.77	
Optical glass (6 types)	2	
Optical lenses	1-10	
Window glass (50 samples)	1-7	

* Glass used in the manufacture of UV lamps.

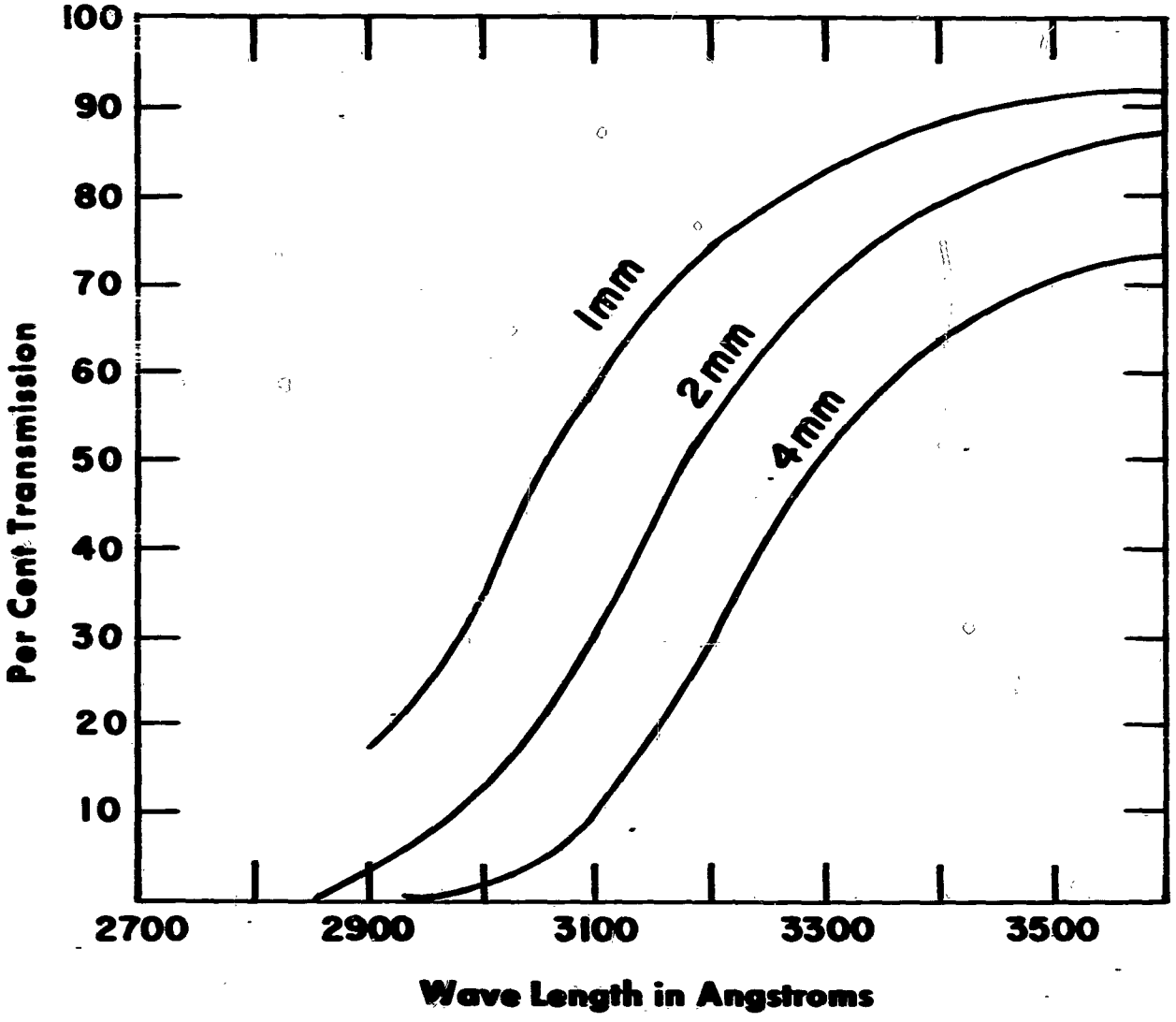


Figure 10. Transmission for Various Thicknesses of Pyrex Clear Chemical Glass No. 774.

TABLE XII. PER CENT TRANSMISSION OF FIVE DIFFERENT UV WAVE LENGTHS THROUGH PYREX GLASS

GLASS	WAVE LENGTHS				
	2537A	3022A	3130A	3342A	3650A
Sheet, 3 mm thick	0	21	47	79	89
Sheet, 4.85 mm thick	0	7	30	69	89
Pebbled sheet, 3 mm thick	0	23	44	73	82
Pressed sight, 5.8 mm thick	0	7	29	72	89
Relative bactericidal effectiveness of wave length	100%				.025%

Plexiglass and other clear plastics transmit very little 2537A energy. The degree of transmittance is a function of the thickness of the plastic as well as the type. Figures 11 and 12 (174) show the per cent transmittance of various wave lengths through several clear plastics.

Various substituted benzophenones are available (11) which can be included in the formulation of many plastics to absorb radiations between 2000A and 4000A and to prevent the discoloration of plastics caused by UV exposure. The newest of these is 2-hydroxy-4-methoxybenzophenone, known as UV-9, marketed by American Cyanamid Company, Dound Brook, New Jersey.

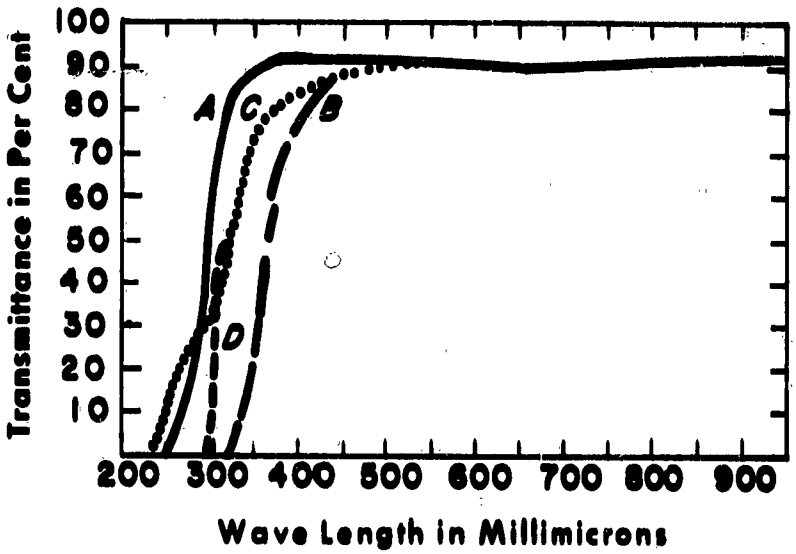
UV radiation of 2537A and longer show high transmission through distilled water. Shorter wave lengths show increasingly higher absorption rates. Koller (174) gives the following table for the transmission of 2537A ultraviolet in distilled water:

Depth in inches	3	6	12	24
Per cent transmission	92	88	78	61

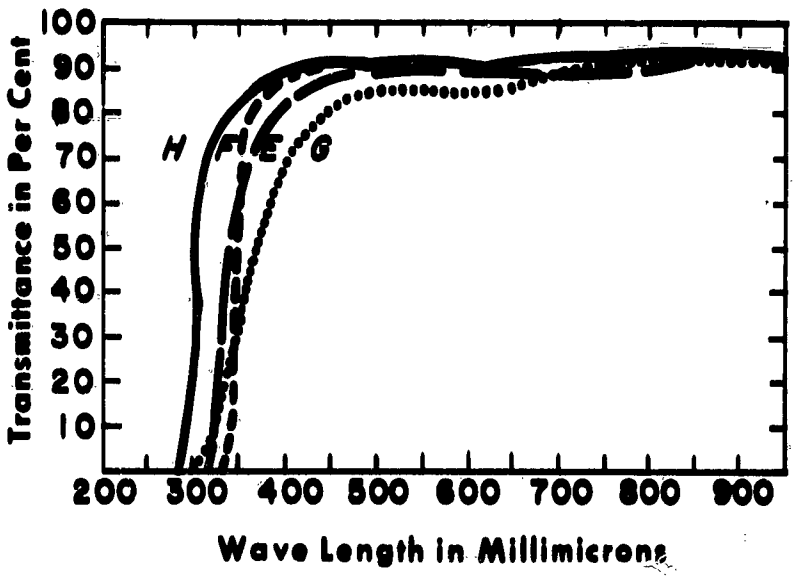
Transmission is reduced by the presence of dissolved multivalent salts or organic matter. Generally, iron salts and organic matter have a greater effect on absorption than do alkali salts. The transmission, t, varies exponentially with the depth of the water, d.

$$t = e^{-ad}$$

The value, a, is called the absorption coefficient. The transmission of 2537A radiation through water of various coefficients of absorption is shown in Figure 13. Municipal waters show a considerable variation in the transmission. Water samples from 19 large U.S. cities transmitted from



A. Methyl methacrylate, thickness 1.47 mm.
B. Allyl alcohol, thickness 3.05 mm.
C. Cellulose acetate butyrate, thickness 0.41 mm.
D. Cellulose acetate, thickness 0.50 mm.



E. Cellulose nitrate, thickness 0.80 mm.
F. Cellulose propionate, thickness 0.23 mm.
G. Ethyl cellulose, thickness 0.79 mm.
H. Polystyrene, thickness 0.45 mm.

Figure 11. Spectral Transmittance of Several Clear Plastics. Koller (174)

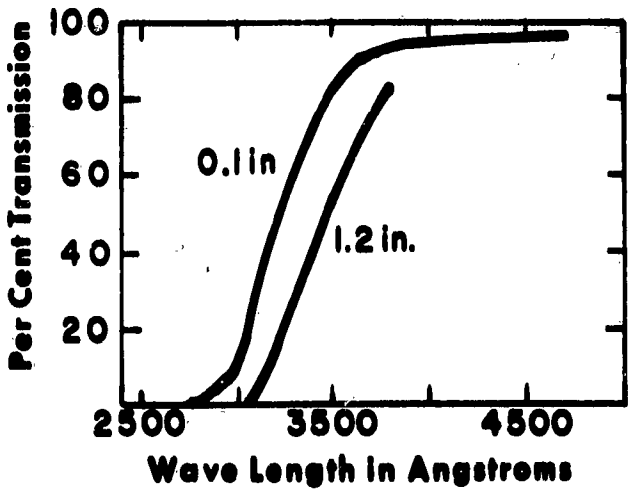


Figure 12. Transmission of Plexiglass.

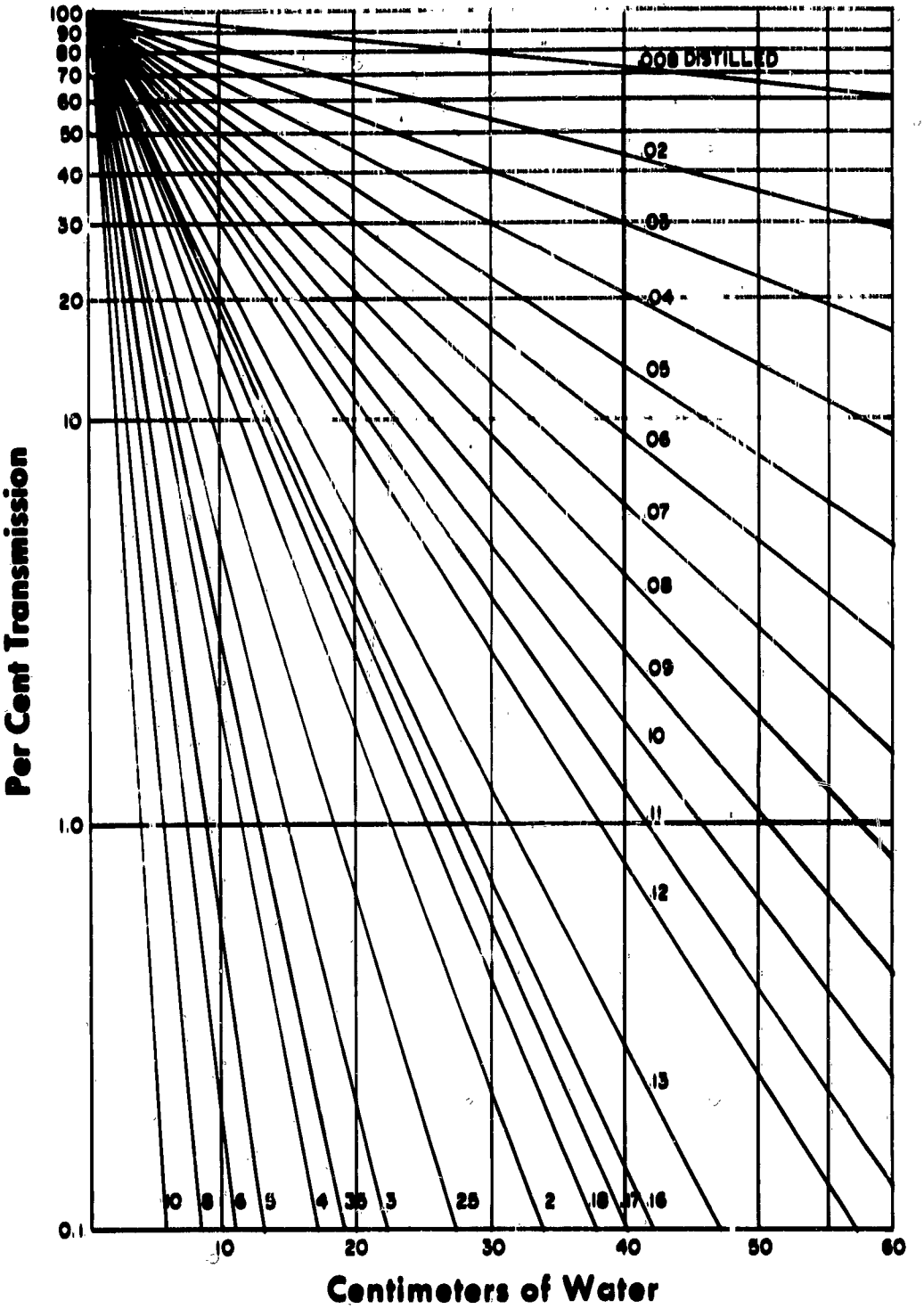


Figure 13. Per Cent Transmission of 2537A for Waters of Different Coefficients of Absorption.

0.36 to 45 per cent of the UV radiation passed through a test cell one foot in depth (186). Contamination of water with small bits of filter paper, cork, or dust can reduce UV transmission by a large factor. The transmission of distilled water can be reduced from 95 to 58 per cent by passage through two filter papers.

Ronge (258) reviewed information on the permeability of clothing to UV radiation. Whipcord fabrics are practically impermeable to UV radiation, while cotton or silk stocking material may transmit from 18 to 40 per cent. The pore-size or weave of the fabric has the most effect on UV penetration. Hart (124) states that closely woven starched cloth is sufficient to stop over 99 per cent of the radiation from a bactericidal lamp.

B. EXPERIMENTAL

Studies have been made by the authors on the transmittance of 2537A energy through various materials using a 15-watt, hot cathode germicidal lamp. The UV intensity at a point two inches from the outer edge of the lamp, as measured by SM-200 click meter, was 1710 microwatts per square centimeter.

As shown in Table XIII, only the onion skin paper, the powdered weighing paper and the operating gown showed detectable transmission of the germicidal radiation. No reading was obtained on the instrument with the other materials tested.

In general it may be concluded that most surfaces, including glass, plastics, and paper, are incapable of transmitting 2537A radiation to any great extent. Laboratory glassware (soft glass or Pyrex) does not transmit the 2537A band. Pure distilled water three to six inches in depth does not seriously reduce UV penetration, while municipal waters may absorb a considerable proportion of radiation.

TABLE XIII. TRANSMISSION OF 2537A RADIATION THROUGH VARIOUS MATERIALS

Intensity directed against each material = 1710 microwatts per square centimeter

MATERIAL	MICROWATTS PER SQ CM PASSING THROUGH MATERIAL	PER CENT TRANSMISSION
Flexible vinyl plastic - 2 mill thick	0	0
Plastic visor of face shield	0	0
Plexiglass, ¼" thick	0	0
Visor of plastic personnel hood	0	0
Back of plastic personnel hood	0	0
Pyrex Petri dish	0	0
Glass eye - piece of gas mask	0	0
Brown wrapping paper, sheet	0	0
Bond typing paper, 1 sheet	0	0
Scratch paper, 1 sheet	0	0
Onion skin paper, 1 sheet	27	1.5
Powder weighing paper, 1 sheet	73	4.2
Bath towel	0	0
Operating gown	20	1.2

VI. MEASUREMENT OF UV RADIATION

A. UNITS OF MEASUREMENT

Just as erythemal energy is radiant energy capable of producing erythema, germicidal energy is capable of producing germicidal effects. Visible radiant energy, on the other hand, is capable of producing luminosity. These energies may be measured and expressed in terms of erg-seconds, joules, microwatt-seconds per square centimeter (sq cm), or lumens.

In the case of erythemal producing radiation, the flux is analogous to light or to luminous flux. Flux is energy measured according to the effectiveness of equal amounts of energy of various wave lengths. Luminous flux, or brightness, is measured in foot-lamberts and the corresponding term for use with erythemal UV is E-viton, or microwatts per square centimeter of effective radiation. Thus, the unit of exposure is the E-viton-second per square centimeter which is equivalent to ten microwatt-seconds per square centimeter of 2967A radiation or equivalent.

In the measurement of germicidal energy, the radiation emitted by the lamps is concentrated in the spectral region of 2537A which is near the maximum for germicidal effectiveness. The intensity of germicidal radiations is usually expressed in microwatts per square centimeter of 2537A radiation and is a measurement of flux. The total UV dose is calculated as the product of the flux, the exposure surface (1 sq cm), and the exposure time, thus giving microwatt-minutes per square centimeter. This term represents the total energy incident upon a square centimeter of surface and for simplicity may be designated as the ET (intensity x time) value.

The following physical definitions are helpful when converting or calculating UV intensities (185).

<u>Energy measurements</u>

1 erg = 1 dyne cm

10^7 erg = 1 joule

= 1 watt-second

<u>Power measurements</u>

1 watt = 10^7 ergs per second

= 10^6 microwatts

1 erg per second = 10^{-1} microwatts

Intensity of radiant power (per unit area)

1 microwatt per sq cm = 10 ergs per second per sq cm

Exposure or dosage

1 microwatt-second = 10 ergs

1 microwatt-minute = 600 ergs

Intensity of exposure or dosage (per unit area)

1 microwatt-second per sq cm = 10 ergs per sq cm

1 microwatt-minute per sq cm = 600 ergs per sq cm

Wells (305) advocated the use of a survival-ratio called a "lethe". One lethe is equivalent to the reduction in bacterial air count resulting from one complete air change in an occupied area. This ratio has been termed a "unit lethal exposure" by Luckiesh (185). The survival-ratio is the fraction of the original concentration (PO) of bacteria per unit area or per unit volume surviving after a given exposure. With certain microorganisms the relationship between the exposure dose and the survival-ratio holds approximately over a range sufficient for practical purposes. The relationship for air-borne bacteria exposed to UV radiations is represented by the following formula:

$$\frac{P}{Po} = e^{-KEt}$$

where Po = initial concentration

P = concentration at time t

e = base of the natural system of logarithms (approximately 2.718)

E = intensity of germicidal flux

t = the time

K = a constant depending on environmental conditions such as humidity

When the exponent of "e" is less than unit, the survival-ratio equals 0.368 and corresponds to a survival of 36.8 per cent, or one lethe. The established procedure is to plot a straight line survival curve using a logarithmic ordinate scale. In some instances where the required accuracy is not critical, the use of this system may be advantageous, but in general it

is better to calculate inactivation of microorganisms based on the radiation dose available to inactivate and experimental data showing the relative resistivity of various air-borne forms.

The most convenient method to express the concentration of UV radiation is by the use of the term ET (microwatts per sq cm x time in minutes). This tends to make the experimental data presented here more easily applicable for practical use. For example, in one test room (375 cu ft) two 30-watt, hot cathode, UV lamps without reflectors located on the ceiling were turned on and a series of readings taken with a meter to measure the intensities falling on various surfaces. An average value was determined for each surface as shown in Table XIV.

TABLE XIV. UV INTENSITIES AT VARIOUS DISTANCES FROM TWO 30-WATT LAMPS

SURFACE	DISTANCE FROM CEILING, cm	MICROWATTS PER SQUARE CENTIMETER
On top of transfer cabinets	90	85
On table tops	180	25
On chair tops	210	18
On floor	266	15

From these data one can determine that ET value for any surface level by multiplying by the expected exposure time. Then with this figure and other data presented here, an estimation of the conditions necessary to obtain any degree of microbial killing can be made. Of course, in actual practice it is best to include a suitable safety factor.

B. METHODS OF MEASUREMENT

Because of the characteristics of the spectral output of the low-pressure mercury vapor UV lamp, the problem of measurement is greatly simplified. Since over 95 per cent of the energy emitted from the lamps is in the range of 2537A, a measuring device having a good sensitivity in this region without high selectivity of response is satisfactory for making accurate intensity measurements. In other words, practically all that is necessary is to compensate in some manner for the visible radiations and for the UV radiations longer than about 3000A. Two general methods for the measurement of energy in the region of 2537A are in common use.

The first method involves the use of a photocell or photronic cell which measures, through quarts, the total UV radiation plus white light. Then by means of a suitable filter, such as a thin sheet of Pyrex glass, radiations longer than 3000A can be measured and subtracted from the total. The Pyrex glass transmits the energy in the 3000A region and longer but not the 2537A energy, whereas the quarts is transparent to both. The difference in the two regions is designated at 2537A radiation.

Such a meter developed by Taylor (280) consists of a light meter (photronic cell graduated in foot-candles) and a special attachment. The attachment employs a thin layer of fluorescent material between a sheet of quarts and a sheet of glass. Zinc-silicate phosphor was selected as the fluorescent material because of its selective character when exposed to UV radiation of various wave lengths. The maximum sensitivity of zinc-silicate phosphor occurs at 2537A (99 per cent), while radiations of 3000A and longer produce no fluorescent effect. In the operation of the meter a reading is first taken with the quarts and then with the glass side of the attachment exposed to the UV source. The difference in the two readings is proportional to the intensity of the UV energy. Each attachment is calibrated to give a factor by which the difference in the two readings (in foot-candles) must be multiplied in order to obtain the intensity in microwatts per square centimeter. The sensitivity of the meter is such that one foot-candle on the meter indicates an intensity of about 40 microwatts per square centimeter of energy in the 2537A range. This meter is suitable only for measuring high intensities received at a short distance from the UV source. The meter is called the Luckiesh-Taylor Germicidal Attachment and is available from General Electric Company.

Westinghouse Electric Corp., manufactures a similar meter, designated as the SM-600 UV meter, for the purpose of measuring the intensity of UV lamps. This meter is a converted foot-candle meter with enclosed filters which is held directly against the UV lamp to obtain the intensity. Correction factors are supplied so that readings may be taken from all types of UV lamps.

In Sweden, Laurell and Ronge (179) constructed a "filter difference" meter using a selenium barrier-layer cell with its lacquer surface removed. Activation of the cell is measured on a galvanometer. A reading is first made through an UV-transparent but light-absorbing UG-5 filter. A second reading is made using 0.20 per cent copper sulfate solution or a thin layer of cellulose acetate as an additional filter to remove bactericidal radiation. The difference in the two readings is a measure of the UV intensity.

Other meters have been designed using phosphor-coated quarts and Pyrex glass cells connected to microammeters or galvanometers to measure the difference in current generated by the two cells. The sensitivity depends upon the selection of the phosphor cells. Calibrations are generally such that one microampere is equal to an intensity of ten microwatts per square centimeter of 2537A energy.

The second general method for measuring germicidal UV radiations involves the use of phototubes.

Several types of vacuum phototubes for measuring UV intensities are available. These phototubes operate on the principle that a pure metal has photoelectric response to a definite band in the spectrum. The cathode surfaces are coated with a metal which is sensitive in the desired range. The phototubes are made of UV transmitting glass. Perhaps the first tube of this type was developed by Koller (173) who used a cadmium-magnesium alloy as the cathode in a Corex D bulb. The upper response of this bulb was around 3400A.

The characteristics of four UV phototubes made by Westinghouse Corp. (310) are shown in Table XV. The zirconium tube has its maximum response at 2340A, the thorium phototube at 2550A, the tantalum tube at 2400A and the platinum tube at 1849A. The lower limit of response (2000A) is the same for the first three and is determined by the glass of the bulb. The limit of upper response is a function of the metal used. For measuring germicidal radiation, the WL-775 tantalum phototube is probably preferable because radiation longer than 3000A will not sensitize the tube. This means that the WL-775 tube is practically insensitive to ordinary sunlight, Figure 14. The manufacturer recommends that phototubes be cleaned with an alcohol soaked cotton pledget before use. Phototubes are presolarized at the factory before being calibrated. However, exposure to high intensity radiation can cause further solarization. Phototubes must be used with electrometers or integrating meters such as the Westinghouse SM-200.

TABLE XV. CHARACTERISTICS OF WESTINGHOUSE UV PHOTOTUBES
(Westinghouse Electric Corp. 310)

DESIGNATION	WL-767	WL-773	WL-775	WL-789
Cathode surface	Zirconium	Thorium	Tantalum	Platinum
Maximum response	2390A	2550A	2400A	1849A
Response range	2000A-3150A	2000A-3677A	2000A-3000A	1700A-2100A
Uses	Erythemal Vitamin D	Erythemal Vitamin A Vitamin D General UV	Erythemal Bactericidal	Ozone

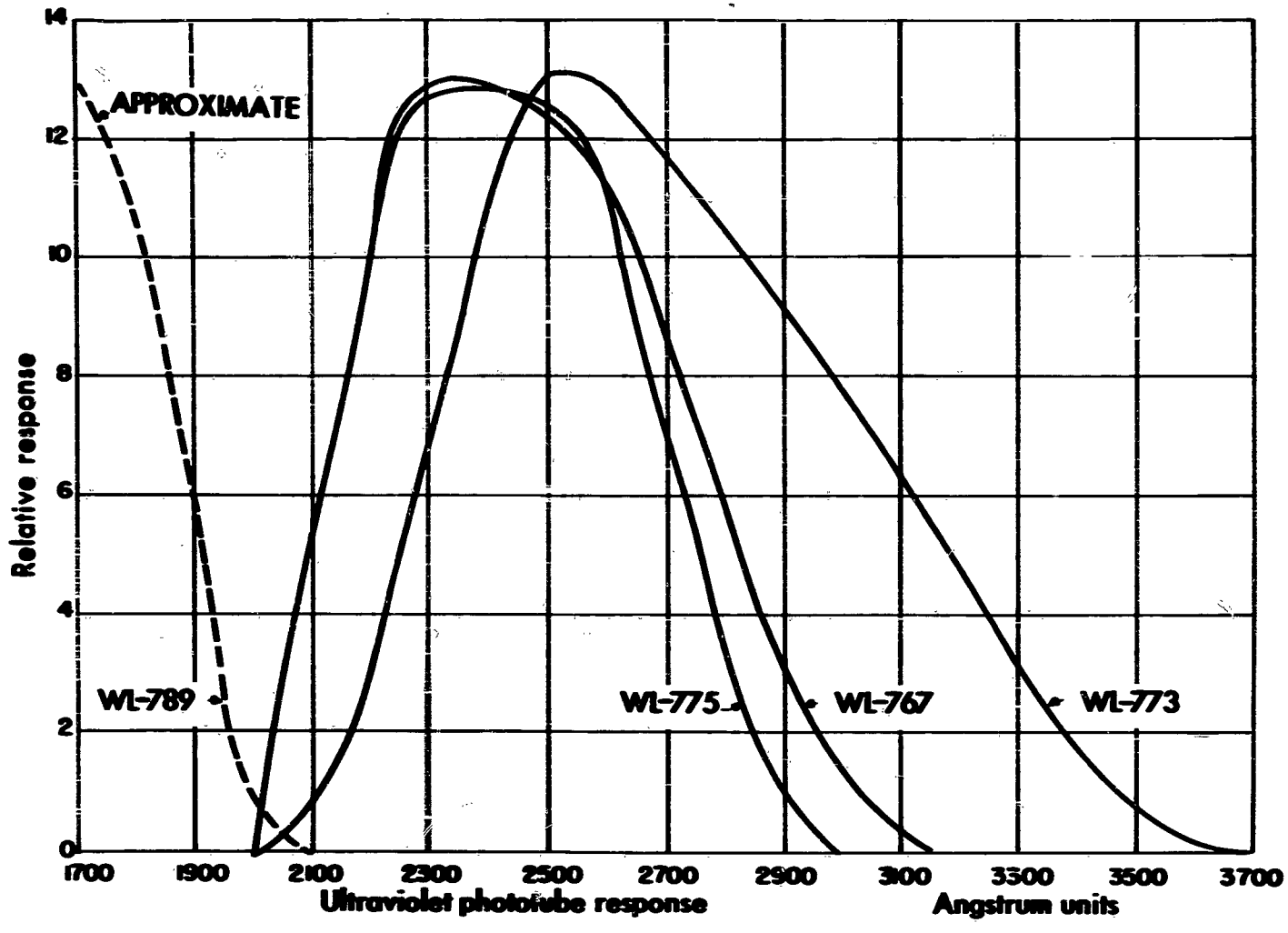


Figure 14. Response of UV Phototubes.

An UV meter using the WL-775 phototube was used in many of the studies reported here. The meter was capable of accurately measuring intensities from one to 150 microwatts per square centimeter. The readings were obtained in milliamperes and then converted to microwatts per square centimeter by applying the proper correction factors. This meter was standardized by the National Bureau of Standards before being used.

Another satisfactory meter for measuring intensities of 2537A ranging from one to 500 microwatts per square centimeter was designed and built for the authors by the local engineering department. Information for the basic design was obtained from Luckiesh (185). The meter utilizes an RCA type 935 vacuum phototube employed in combination with a suitable amplification system. The phototube has a low cutoff of approximately 2000A. A filter is used to zero the instrument, and an ordinary glass microscope slide is used to eliminate any reading due to luminous flux. The meter is first adjusted to zero with the glass slide covering the aperture. Consequently, when the microscope slide is removed, any reading on the meter will be due to radiant energy in the region between 2000A to 2650A. This meter was also standardized by the National Bureau of Standards, and has proved valuable in practical as well as experimental studies. The wiring diagram is shown in Figure 15.

A battery operated "Germicidal UV Intensity Meter" is manufactured by General Electric Company. The portable meter utilizes a phototube and an Eveready #467 battery. Intensity measurements are expressed in milliwatts per square foot, which can be changed to microwatts per square centimeter by multiplying by 1.075. The meter has four sensitivity selectors and can measure intensities up to 100 milliwatts per square foot.

The Archer-Reed Company, Dearborn, Michigan, has developed an intensity meter with a tantalum photocell for the measurement of low intensity germicidal radiation.

A meter for determining the per cent reflectance of UV radiation from flat surfaces such as walls and ceilings has been made by General Electric Company (281). The meter utilizes a four-watt germicidal lamp as the radiation source.

An integrating UV meter which records the energy by means of a mechanical counter and an audible click device is manufactured by Westinghouse Electric Corp. This meter is known as the SM-200 UV meter. The circuit diagram of this meter is shown in Figure 16. Westinghouse Electric Corp. (311) has supplied the following descriptive information.

UV radiation on the sensitive surface of the phototube causes current proportional to the intensity of the radiation to flow through the phototube and charge condenser C_1 (Figure 16). When the voltage across the condenser reaches a definite value, the condenser discharges across the trigger T and cathode K, electrodes in the relay tube WL-759. This action starts a discharge between cathode K and anode A which operates relay L_1 in meter case by discharge of condenser C_2.

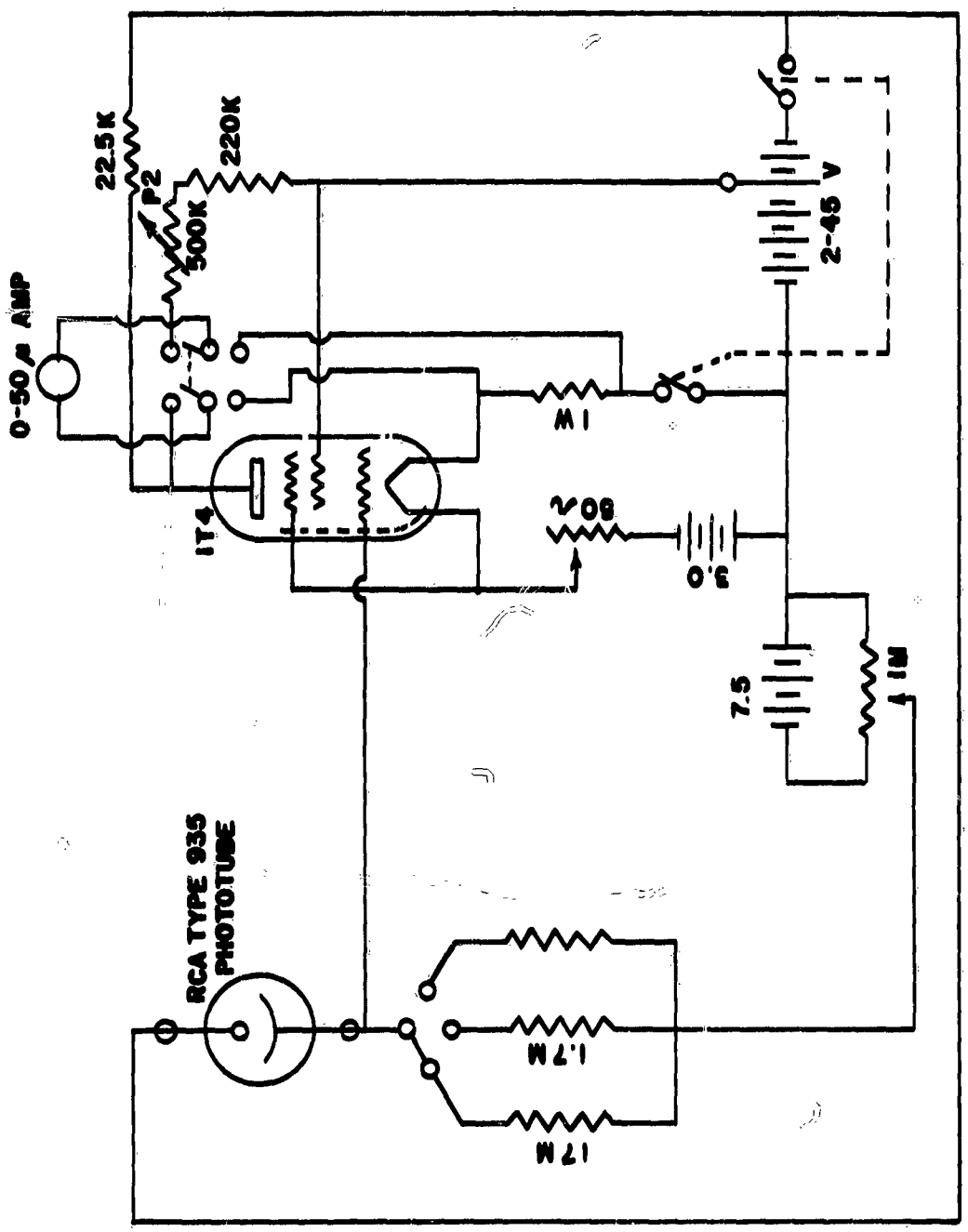


Figure 15. Wiring Diagram-UV Meter.

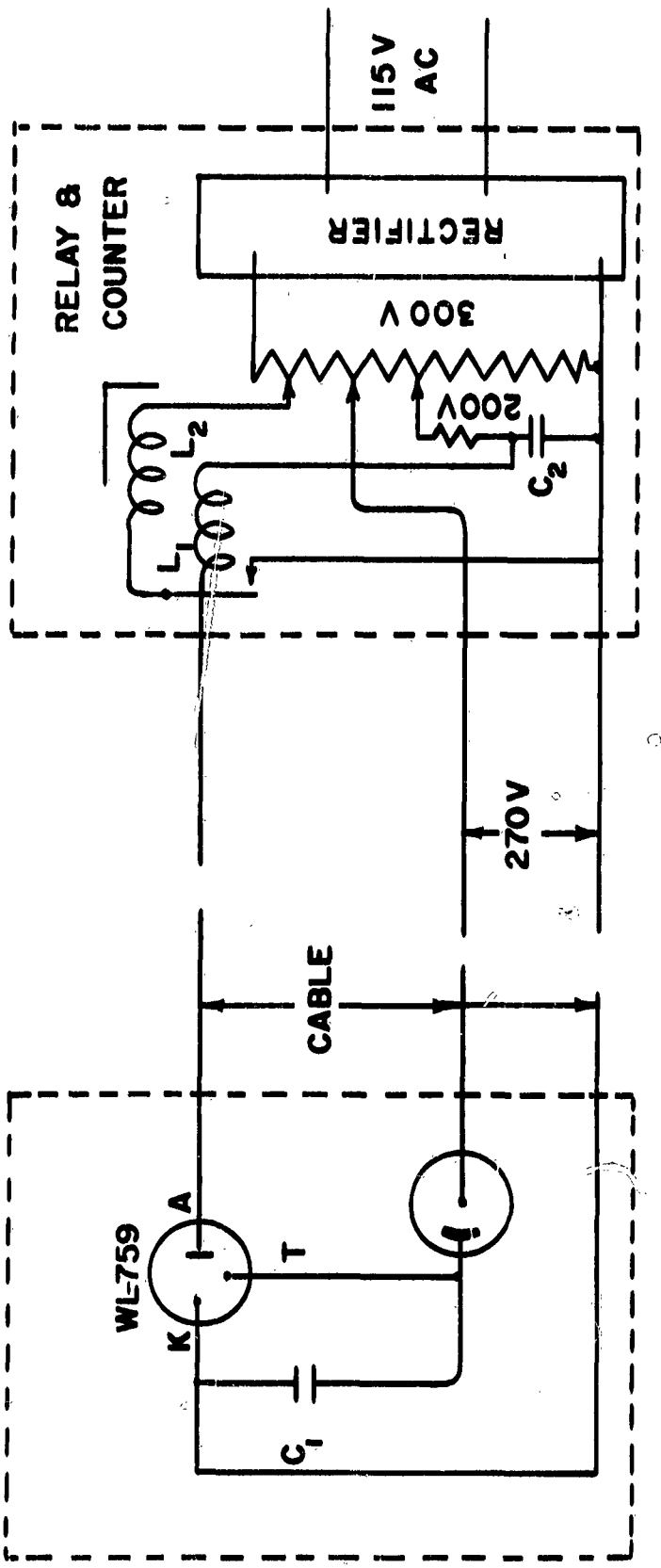


Figure 16. Circuit Diagram of the Westinghouse SK-200 UV Meter.

The momentary closing of the relay L_1 contacts operates the counter L_2. As soon as condenser C_2 is discharged, the current through the relay tube between anode A and cathode K ceases to flow, and the circuit is in condition to repeat the cycle of operations. Because of the lag in the operation of relay L_1 and counter L_2 the meter will not be accurate at intensities which give counter clicks of more than 40 per minute. To insure accuracy of measurement and long life of the relay and counter, it is recommended that the phototube be placed at such a distance from the source being measured that the counter on the meter will operate at approximately 20 clicks per minute.

The characteristics of phototubes and relay tube WL-759 vary, hence it is necessary to calibrate the SM-200 meter for each tube used. If a phototube or relay tube is changed, the meter should be returned to the factory to be recalibrated with the new tube because this cannot be done conveniently in the field.

An SM-200 meter registers one unit on the counter, or one click, for a specific number of microwatt-seconds. Usually one click equals between 250 to 300 microwatt-seconds and the meter maintains its calibration over long periods of time if used carefully. The sensitivity of the phototube and the capacity of the control condenser must be balanced to keep the number of clicks per minute within the maximum of 40 per minute. More than 40 clicks per minute may cause inaccurate readings and the counter to be temporarily inoperative.

The SM-200 meter can be calibrated for use with any of the UV phototubes listed in Table XV. The WL-775 tantalum photocell is the photocell of choice for use with artificial UV radiations. This meter has been found to be the most accurate of those discussed. In practice the meter is used periodically to standardize other meters routinely used.

C. CALCULATING UV INTENSITIES

The method of calculating the total output of UV lamps has been supplied by Nagy (218). The formula used is:

$$W = \frac{K \times L \times D^2}{10^6}$$

where W = watts of germicidal UV radiation (2537A),

K = a constant which is 9.92 for 30-inch lamps,

L = an intensity measurement in microwatts per sq cm at a perpendicular distance from the lamp, which is at least five times the length of the lamp, and

D = that distance from the lamp, in centimeters.

For example, if the intensity, 300 centimeters from a 60-centimeter lamp, is 10 microwatts per square centimeter:

$$W = \frac{9.92 \times 10 \times (300)^2}{10^5} = \frac{88.28 \times 10^5}{10^6}$$

$$W = 8.82$$

For use in conjunction with the above formula, a curve such as shown in Figure 17 is conveniently used to determine average intensities at various distances from the source.

It is sometimes useful to calculate the UV intensity at the surface of a lamp or at distances very close to the lamp surface. First the total output must be determined. This figure may be obtained by the method described above. Then the area of the emitting surface is calculated, remembering that the effective emitting length of the tube must be used rather than its over-all length. If the area is divided into the total output, an estimate of the emission intensity per unit area of lamp surface is obtained. For example, the effective emitting area of 17-watt cold cathode lamps is approximately 383 square centimeter and the total output is 5.2 watts. Thus, dividing 5.2 by 383 yields 0.01361 watts per square centimeter or 13,610 microwatts per square centimeter of bulb surface.

This technique can also be used to estimate intensities up to several inches from the lamp surface. Thus one could calculate the intensity per unit area of a cylindrical type 10 centimeters in diameter into which a UV lamp had been centered by determining the area of the tube and dividing into the total output.

A "factor system" is sometimes useful to estimate the approximate radiation intensity on surfaces at different distances from an UV lamp. The intensity of the lamp at one meter, in microwatts per square centimeter is multiplied by a factor for the distance selected. These factors are shown in Table XVI. They are applicable for hot and cold cathode lamps as well as Slimline lamps. The intensity at one meter can be measured by an SM-600 meter or it can be taken as the 100 hour output. Since it accounts for lamp deterioration and for the output at the prevailing temperature, the former method is probably the more accurate. The intensity factors in Figure 17 are applicable only for UV lamps with no reflectors. These factors provide an easy method for quickly calculating UV intensities within five per cent accuracy. For precise measurements, however, a reliable meter should be used.

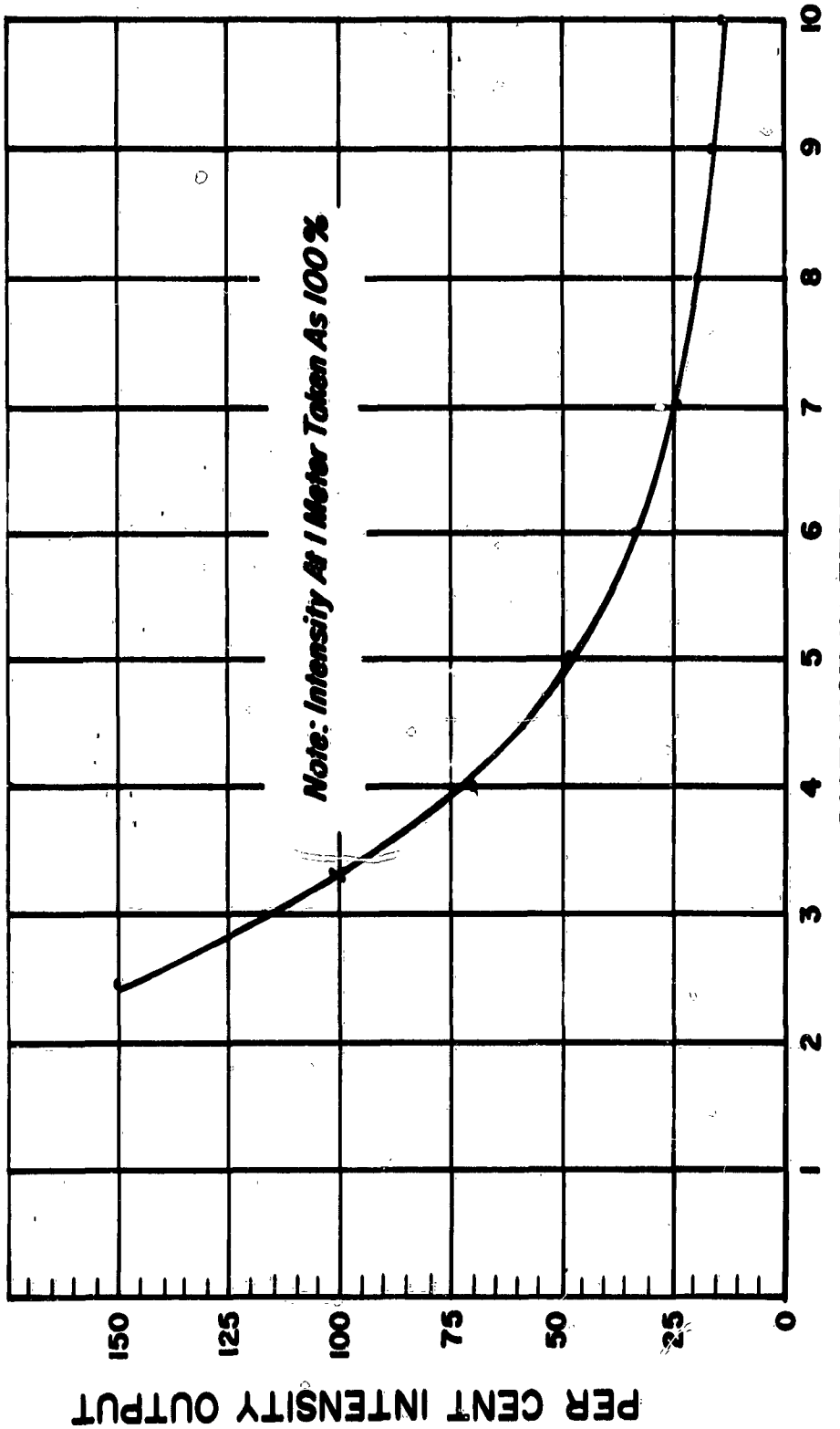


Figure 17. Intensity vs Distance from Lamp for Slimline and Cold Cathode 17-Watt Lamps.

TABLE XVI. FACTORS FOR ESTIMATING GERMICIDAL INTENSITIES AT VARIOUS DISTANCES FROM AN UV LAMP

DISTANCE FROM LAMP, inches	INTENSITY FACTOR
2	32.3
3	22.8
4	18.6
6	12.9
8	9.85
10	7.94
12	6.48
14	5.35
18	3.6
24	2.33
36	1.22
39.37 (1 meter)	1.00
48	.661
60	.452
80	.256
100	.169
120	.115

VII. OZONE

The role of ozone in any adequate discussion of UV radiation must be considered because this gas is produced by some germicidal lamps. A review of a limited number of publications available concerning ozone indicates that controversial issues exist. It is appropriate, therefore, that this chapter include a rather complete discussion of ozone as it pertains to UV radiation.

Because the reports are numerous and conflicting, a certain amount of selectivity has been necessary in the choice of reference material. Reference material and original studies which were supplied by Dr. R. Nagy (219, 220,221) were invaluable. A report and bibliography by Thorp (285,287) was also very helpful and should be referred to for more complete information.

A. THE CHEMISTRY AND PHYSICS OF OZONE

In 1782, Cavallo noted that "electrified air" exhibited a purifying action on decaying animal and vegetable matter. Over 50 years later, in 1840 Schonbein showed that the compound was a form of oxygen and named it ozone.

Ozone is a gaseous compound produced by the photochemical reaction of $3\ O_2$ to $2\ O_3$. It is a powerful oxidising agent and readily combines with many substances. It is a deep blue liquid at -180°C. When ozone oxidizes materials, oxygen is released. Ozone is more soluble in water than is oxygen.

Some of the constants of pure ozone have been listed by Hann and Manley (115).

B.P.	-112°C
M.P.	-250°C
d_4 -183	1.571
Vapor density at 0°C, gms/l	2.14
Solubility in water at 20°C, gms/l	0.57

For concentrations of ozone below 1.0 per cent, values should be expressed in terms of ppm/wt. Table XVII (285) will be found valuable for calculating and converting ozone concentrations.

Ozone can be used to hasten the drying of paints, oils, and varnishes, by rapid oxidation. It is employed to sterilize and deordorize water, bleach organic pigments, and oxidize organic odors (221). For filtering large amounts of ozone from breathing air, Van Atta (291) has recommended a bed of absorbent carbon or possibly silica-gel.

TABLE XVII. OZONE CONCENTRATION FACTORS

0°C - 760 mm
1 liter of ozone weighs 2.144 gm
1 liter of oxygen weighs 1.429 gm
1 liter of air weighs 1.293 gm

To convert:

Parts per million to per cent, divide by 10,000 (1)
Parts per million by volume to parts per million by weight:
in oxygen, multiply by 1.50 (2)
in air, multiply by 1.66 (3)

NOTE: If over 10,000 parts per million for accuracy convert first to per cent and then use equations (8) or (9).

Milligrams per liter to parts per million by weight:
in oxygen, multiply by 700 (4)
in air, multiply by 773 (5)

NOTE: If over 10 mg/l for accuracy convert directly to per cent by means of equations (6) or (7).

Grams per liter (X) to weight per cent, use the following equations:

in oxygen,

$$\text{Wt per cent} = \frac{(X)\ (100)}{1.429 + (.334)\ (X)} \qquad (6)$$

in air,

$$\text{Wt per cent} = \frac{(X)\ (100)}{1.293 + (.397)\ (X)} \qquad (7)$$

Volume per cent (Y) to weight per cent, use the following equations:

in oxygen,

$$\text{Wt per cent} = \frac{(Y)\ (214.4)}{142.9 + (.715)\ (Y)} \qquad (8)$$

in air,

$$\text{Wt per cent} = \frac{(Y)\ (214.4)}{129.3 + (.851)\ (Y)} \qquad (9)$$

Thorp (285).

The diffusion rate of ozone into air is about 0.12 centimeter per second. The half-life of ozone at room temperatures and at relative humidities between 54 and 88 per cent is approximately three minutes (78). Moisture and temperature affect the rate of decomposition of ozone. The rate of decomposition is proportional to the square root of the absolute humidity. A twofold increase in the decomposition rate would result from a relative increase from 20 to 90 per cent (at room temperature). This is a fivefold increase in absolute humidity. At lower temperatures, moisture affects decomposition to a lesser degree because the absolute humidities are much lower. The decomposition rate of ozone is increased by a rise in temperature. When the temperature is raised from 7°C to 32°C, the equilibrium concentration is reduced one-half (78). At a temperature of 100°C the decomposition of ozone would be nearly instantaneous (219). The amount of exposed surface area also affects the rate of ozone decomposition. Ewell (78) states that the decomposition coefficient (K) of ozone is approximately:

$$K = \frac{0.7}{t_{\frac{1}{2}}} = 0.23$$

where $t_{\frac{1}{2}}$ = the ozone half-life (three minutes)

A method for calculating the ozone production of UV lamps is given on page 89. Information on the physical properties have been conveniently compiled by Thorp (287).

B. MEASUREMENT OF OZONE

For the past 97 years, the most common reagent for ozone detection has been potassium iodide solution, although other reagents have been suggested (283).

One simple test involves the use of strips of filter paper which have been dipped in a solution containing starch and potassium iodide (61). Upon exposure to ozone, the paper will turn deep blue. The test is not quantitative, and it is nonspecific in that other oxidising agents (e.g. chlorine) will produce the same reaction. This crude method is sometimes used for estimating concentrations between 0.4 to 20 ppm per volume. It has been used for checking ozone concentrations in cold storage rooms.

In 1940, Thorp (283) reported some improvements in the starch-iodide method of ozone analysis. The common method then in use involved passing ozone containing air through a neutral solution of potassium iodide, acidifying the solution and titrating the free iodine with a standard sodium thiosulfate solution. Using 2 N potassium iodide solution in this manner, ozone concentrations of 0.0013 milligram per cubic centimeter of solution could be detected. At this sensitivity, a minimum of 9.9 liters of air containing 0.1 ppm per weight ozone had to be sampled for each cubic centimeter of potassium iodide test solution. Lowering the pH of the test solution failed to increase the sensitivity but introduced other errors (63).

Thorp (283) found that the use of a buffer solution increased the sensitivity of the potassium iodide solution. Five grams of aluminum chloride hexahydrate and one gram of ammonium chloride made up to one liter constituted the stock buffer solution. Five cubic centimeters of the stock buffer were added to each 100 cubic centimeters of potassium iodide test solution before the test was run. The test soltuion was not acidified during the titration and, on storage, was stable for three hours in light and 40 hours in the dark. Using this method, Thorp increased the sensitivity to 0.00062 milligram of ozone per cubic centimeter of potassium iodide solution. The titration was made with not greater than 0.01 N thiosulfate using a 2-cubic centimeter microburet. Thorp recommends that "an absorption tube containing chromic acid and a tube containing potassium permanganate ..... be provided before the potassium iodide absorption bottles" to insure that only pure ozone reaches the test solution.

Nagy (219) investigated the accuracy of an ozone analysis method in which the potassium iodide was titrated (225) and another (272) employing a colorimetric analysis and found the results of some to be high by as much as a factor of ten.

In 1938, Paneth and Edgar (233) suggested a modified method of ozone analysis with potassium iodide. This method was reported further in 1941 (234) and in 1944 (105), and modified in 1946 by Crabtree (50,51). Crabtree's method has been used by Nagy at Westinghouse Electric Corp. who has supplied a description of the method (219). Since this method appears to be best for accurate determination of small quantities of ozone, Crabtree's description is presented below.

To furnish sufficient iodine for measurement in the short time allotted, a large volume of air must be sampled. To insure absorption of the ozone, the air to be measured is made to generate a fine spray of potassium iodide solution. In this way a large solution surface is furnished for the reaction. The apparatus is shown schematically in Figure 18.

In Figure 18, A, is a glass tube 0.375 inch in diameter and approximately four inches long, terminating at B in a short length of capillary tubing with a bore of one to two millimeters. Concentric within A is a smaller glass tube, C (Figure 18, a, shows this assembly on a larger scale). The end of C is first carefully heated in a blow-pipe flame until the bore is reduced in size so as to just admit a number 69 drill. At this thickened end two flats are ground off on a sheet of fine Aloxite paper as at D in Figure 18, b. When in position in tube A, end D fits snugly against the hole in capillary B. The nozzle C may be sealed to A at the upper end, but it is better to rely on the rubber connection at E to hold the tubes in place, since once sealed in, C cannot be removed for cleaning in the event of blockage.

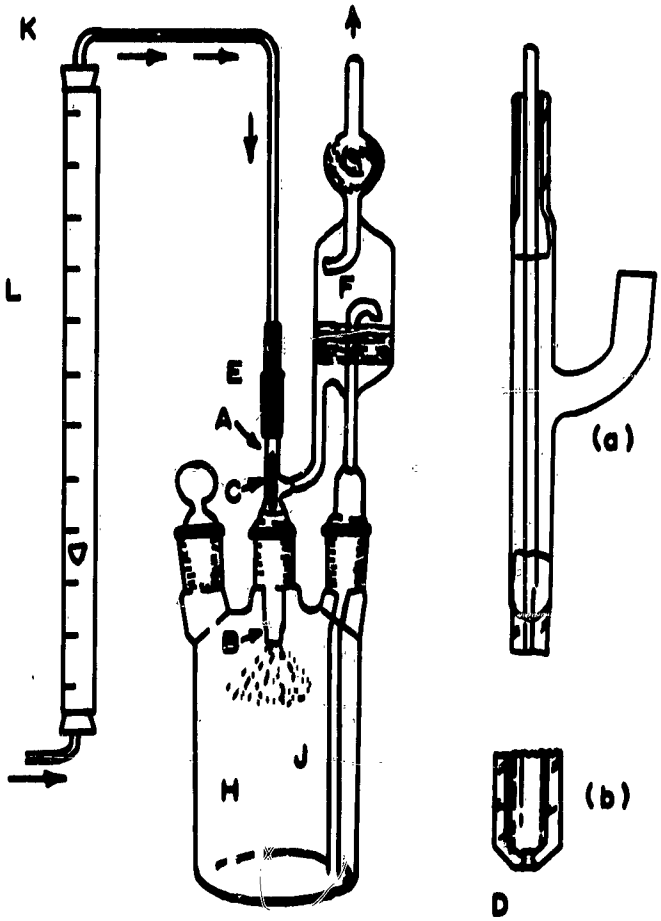


Figure 18. Ozone-Absorbing Device.

A trap, F, is two inches in diameter and four inches long. G is an enlargement in the exit tube 1.5 inches in diameter, containing glass wool to trap spray passing G. The trap F is connected to the side tube of A by rubber tubing or the trap may be permanently attached as shown in the figure. The rubber connector is more convenient, but the tubing used must first be soaked for a long period in dilute iodine solution and thoroughly washed, or iodine may be taken up from the reagent. H is a one-liter, three-necked Woulff's bottle in which A and F are secured by Pyrex ground joints, A occupying the center opening with B protruding just below the neck and tube J reaching to within 0.5 inch of the bottom of the bottle. The third neck serves for introducing and removing the reagent.

A is connected through rubber joints and glass or plastic tube, K, to rotometer, L, graduated from 0 to 1.0 cubic meters of air per hour. The inlet to the rotometer is open to the atmosphere whose ozone content is to be determined. The outlet from G is connected to a vacuum line. After 75 milliliters of reagent are introduced into H, the stopper is replaced, and the vacuum gradually applied. Almost the entire body of liquid will enter F, furnishing a head of reagent at B, where the entering air resolves it into a fine mist which fills the entire bottle. At the end of the run the vacuum is disconnected and the liquid transferred to the titration vessel. When runs longer than one hour are called for, it is necessary to add distilled water at intervals to compensate for evaporation. This is conveniently done through the air intake. The amount of liberated iodine is determined by titration with sodium thiosulfate. Since the amount is so small, 0.002 N to 0.001 N solutions must be used, and since the end point using starch as indicator is uncertain, the electrometric method of Foulk and Bawden (86) is resorted to, in which use is made of the depolarizing effect of iodine on a polarized electrode.

The titration vessel (Figure 19) is a 250-cubic centimeter wide-mouthed extraction flask having a hole in the side near the neck. A two-hole rubber stopper carries into the flask two glass tubes into which are sealed the two electrodes, in this case stout platinum wires (0.1 inch thick) with circular loops at the ends to increase the area exposed to the liquid. Circular loops are used because they are rugged and not disturbed by agitation of the liquid. A potential of 30 to 40 millivolts is applied to the electrodes. This is readily obtained by connecting suitable resistors - e.g., 30,000 and 1,000 ohms - in series across 1.5-volt dry cell and picking off the voltage across the resistor of lower value. A Rubicon 3402 - H.H. with an Ayrton shunt galvanometer is connected in series. A less sensitive type may also be sufficient.

Fifteen grams of potassium iodide are dissolved in 75 milliliters of buffer solution (equal volumes of 0.025 N disodium hydrogen phosphate and 0.025 N potassium dihydrogen phosphate). The solution is introduced into the titration flasks, the electrodes are inserted and the liquid is swirled vigorously over them. Following an initial kick the galvanometer will return to zero if no iodine is present because polarization of the

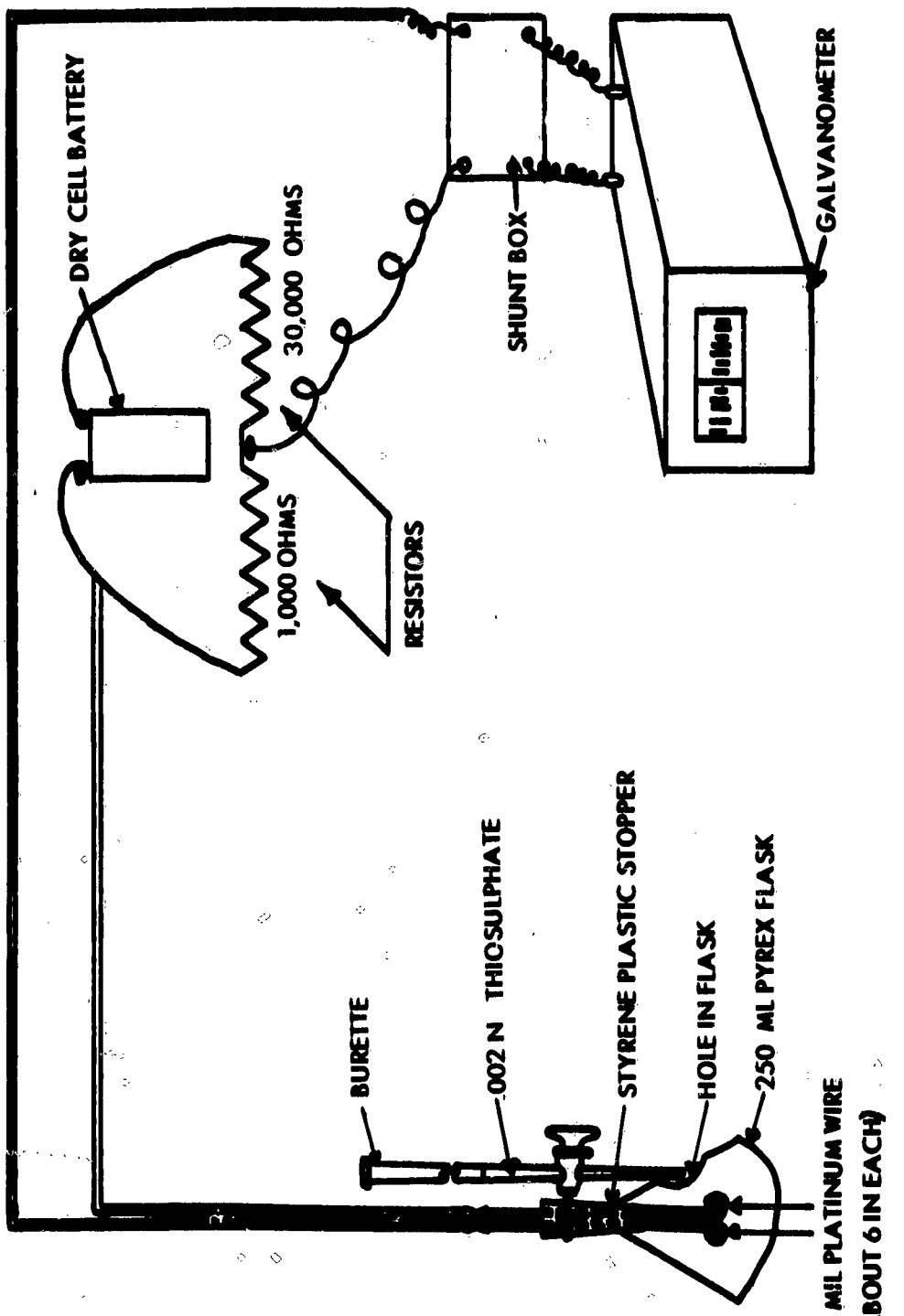


Figure 19. Electrometric Titration Vessel.

electrodes will prevent passage of current. Presence of an oxidizing agent such as iodine removes the polarizing hydrogen from the cathode and current flows. Addition of thiosulfate (through the hole in the side of the flask) until the iodine is removed restores the polarized state and returns the galvanometer deflection to zero. The reagent will usually require the addition of two to five drops of 0.002 N thiosulfate to bring this about.

After the ozone run, the iodide solution containing the iodine is placed in the titration vessel and thiosulfate is added until a barely perceptible yellow remains, then the electrodes are inserted and thiosulfate is added drop by drop at intervals until no deflection is obtained, the liquid being vigorously swirled meanwhile. The liquid is then returned through the trap to rinse the apparatus and the titration is completed. One cubic centimeter of 0.001 N thiosulfate represents 0.0112 cubic centimeter of ozone at standard temperature and pressure.

A little difficulty may be encountered at first in identifying the end point to within one drop of thiosulfate solution at this low concentration. It will be found easier if the titration is made to a small residual deflection of the galvanometer.

Neither the form nor dimensions of the apparatus described are critical. Duplicate apparatus reproduces results within ± five per cent, which is good enough at these low concentrations. It has been found that reducing the concentration of the potassium iodide solution below 20 per cent gives low results. The system described passes about 0.3 cubic meter of air per hour, but this can be controlled by changing the size of the air jet, C. With a given jet there may be considerable leeway in the size of the capillary nozzle. The criterion is to obtain a reaction vessel filled with a mist of reagent.

It is important to remember that potassium iodide in solution is photochemically oxidized to iodine in the presence of light, even in neutral or alkaline solution. Therefore, titration must not be conducted in bright daylight and, during the ozone test, the absorption apparatus must be enclosed in a dark box.

The 0.002 N thiosulfate solution should be standardized at frequent intervals, because dilute solutions of thiosulfate lose strength through oxidation. Since iodine is volatile, some will be vaporized and carried away in the exhausted air, therefore, a correction must be applied to the result obtained. The per cent loss with time is shown in Figure 20.

Investigations by various workers (21,51,105,219) indicate that in the method described very little interference due to other gases or oxidizing agents in the air is to be expected.

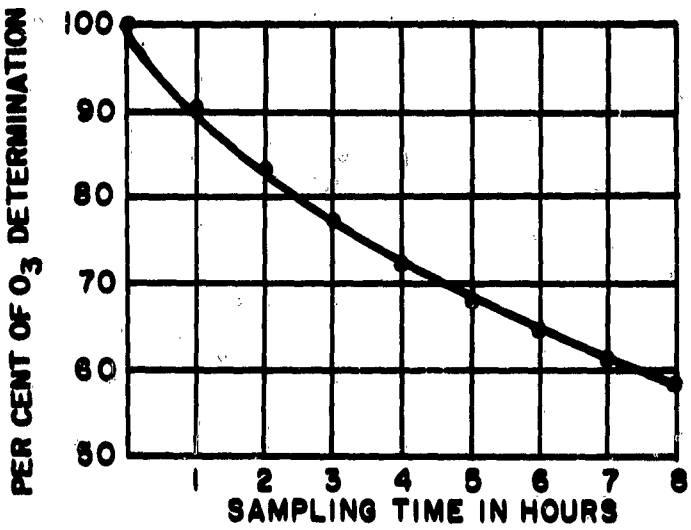


Figure 20. Correction Curve for Ozone Estimation.

A simple ozone meter utilizing a rubber band and a calibrated scale has been developed (37,52). When the rubber band is exposed to minute amounts of ozone, it loses its elasticity. The rate of loss of elasticity gives a measure of the amounts of ozone in the air. An ozone concentration as low as 0.01 ppm by volume can be estimated by this method. This ozone-rubber band method, however, requires careful standardization because:

(a) Ozone is not the only oxidizing gas which will attack rubber,

(b) the reaction depends upon the formulation of the rubber, and

(c) the reaction to ozone is a function of the stress placed on the rubber band.

Other methods of analysis of ozone which have been studied from time to time are:

(a) Reaction of ozone with aldehydes followed by oxidation to acids,

(b) photometric methods depending on the action of ozone to create or destroy fluorescence,

(c) reaction of ozone with potassium permanganate, sodium nitrite and other chemicals, and

(d) optical determination of ozone with infrared or ultraviolet radiation.

Methods for ozone determination up to the year 1954 are conveniently outlined in the bibliography by Thorp (287).

C. OZONE IN THE ATMOSPHERE

The presence of ozone in the atmosphere was noted at least 175 years ago. It was identified as an oxygen compound and named ozone by Schonbein in 1840. Short-wave UV radiation in the stratosphere is responsible, by photochemical action, for the formation of atmospheric ozone (49). The reaction is one of equilibrium because UV also catalyses the breakdown of ozone to oxygen ($3\ O_2 \rightleftharpoons 2\ O_3$). The reaction occurs at a height greater than 30 kilometers and requires radiation shorter than 2000A. The final atmospheric ozone concentration depends not only upon the equilibrium reaction, but also on the presence of oxidizable matter suspended in the air and contact with surfaces to cause catalytic decomposition. The total amount of ozone in the atmosphere is said to be equivalent to a layer about 0.21 centimeter thick at STP. Figure 21, from Crabtree and Kemp (51) gives the ozone variation at different latitudes. Since radiation of below wave length 2000A is not found in the lower atmosphere, ozone is produced at high altitudes only, and is brought into the habitable zone by diffusion and convection.

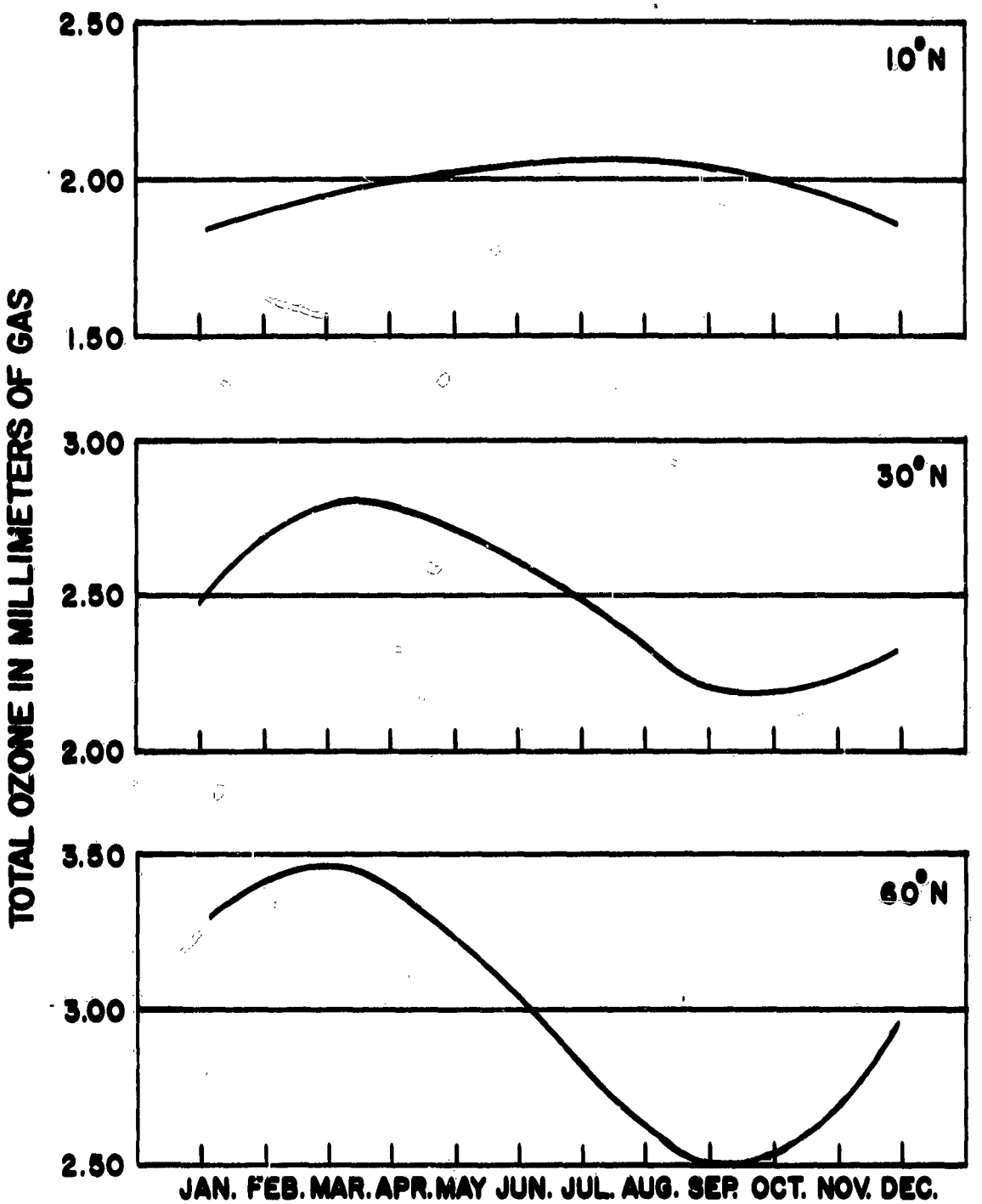


Figure 21. Seasonal Variation of Total Ozone in Atmosphere at Different Latitudes. Crabtree and Kemp (51)

The amount of ozone present in the air at the surface of the earth is known to be quite variable, although precise and accurate information seems to be lacking. Accurate measurements have necessarily depended upon development of adequate methods for sampling ozone (Measurement of Ozone, page 74).

It has been stated that ozone is not present in the air of large industrial cities. Thorp (285) states that this is not true and that appreciable amounts are present. Table XVIII shows average ozone concentrations found in several cities. Figure 22, reproduced from Crabtree and Kemp (51), shows the ozone concentrations at Murray Hill, New Jersey, over a period of one year. The day-by-day irregularity caused by weather conditions is significant. Huntington (157) points out that the normal ozone concentration in some parts of the world frequently exceeds one ppm per weight. In discussing atmospheric ozone, Crabtree and Kemp (51) call attention to the apparent influence of meteorological conditions. High ozone values are found on windy days; low ozone is found on calm days when the winds are predominantly from a westerly direction (in New Jersey). Winds from the East give low ozone concentrations. Highest ozone values are obtained in late summer. There is no difference between the ozone content of the air during the day as compared with night, weather conditions being the same.

TABLE XVIII. AVERAGE ATMOSPHERIC OZONE CONCENTRATIONS

LOCATION	AVERAGE OZONE CONCENTRATION	REFERENCE
Murray Hill, N. J.	0.05 ppm/wt	Crabtree & Kemp (51) 1946
Chicago, Illinois	0.01 to 0.15 ppm/wt	Thorp (285) 1950
Los Angeles, Calif.	0.03 to 0.35 ppm/wt	Bartell and Temple (21) 1952
London, England	0.02 to 0.06 ppm/wt	Stanford Research Inst. (275) 1954
College, Alaska	0.059 ppm/wt	"
Detroit, Mich.	0.026 ppm/wt	"
Los Angeles, Calif.	0.2 to 0.4 ppm/wt (during smog)	"

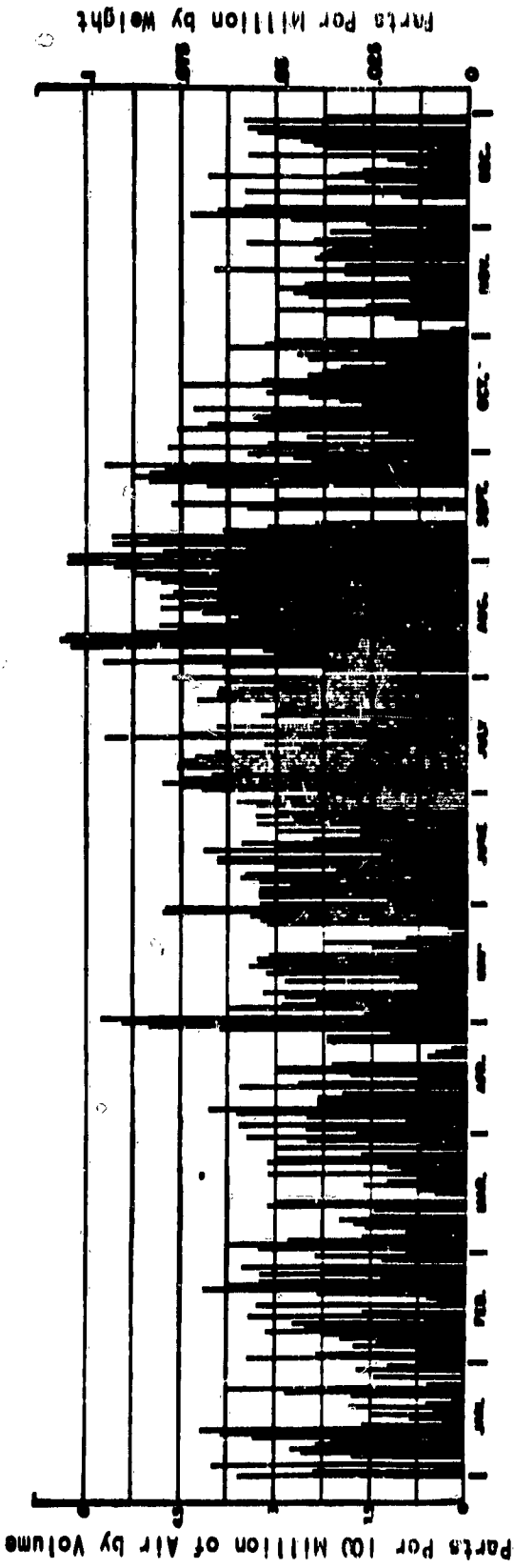


Figure 22. Day-By-Day Content of Ozone in the Atmosphere at Murray Hill, N. J., from January 1 to December 31, 1944.

Reports by Stanford Research Institute (275) and others in connection with the smog problem in Los Angeles county give further discussion of atmospheric ozone.

Summarizing, it may be stated that ozone is present in the air near the surface of the earth, sometimes in concentrations as high as 1.0 ppm per weight, but more often in concentrations from 0.01 to 0.35 ppm per weight or approximately 0.05 ppm per weight.

D. TOXIC LIMITS OF OZONE

Unfortunately, literature dealing with the toxic limits of ozone is extremely inconsistent and rather confusing. However, a clearer understanding of the situation has been made possible in recent years as some of the limitations of earlier work have been explained. The oft quoted introductory remarks of Thorp (285) are appropriate for this discussion.

"Webster defines the word 'toxicity' as 'the degree of poisonness.' To state the toxicity of a substance, however, does not necessarily imply that the substance is a poison inasmuch as all substances are toxic to the human body if taken in excess of normal human tolerance. For example, ordinary table salt is beneficial in small quantities, but an excess, over a long period of time, can produce very harmful results. Arsenic, normally classed as a poison, may also be cited as another example inasmuch as it is often given in small amounts for the sake of certain beneficial reactions. The toxicity of a substance, therefore, must be described with respect to the maximum allowable 'dosage' per unit time. If the human body is capable of eliminating small quantities of a substance within a comparatively short period of time, small dosages of the substance may be tolerated continually even though it may normally be considered a poison. If, however, the body has difficulty in eliminating the substance, toxic effects may result from long exposure to what is normally considered a non-toxic concentration. Ozone falls in the former class of compounds, whereas carbon monoxide may be classed as an example of the latter type substance."

"Gases may also be classed as irritants or non-irritants. Irritant gases are so called because their first reaction is generally on the skin, mucous membrane, or tissues of the nose and throat; whereas non-irritant gas usually requires absorption in the lungs before toxic results appear. In general, it may be stated that the non-irritant gases are more dangerous because the toxic limit may be exceeded before a physical result on the human body is noticeable. Boron trichloride, chlorine, dimethyl ether, ethylamine, ethylene oxide, and ozone may be given as examples of various degrees of irritant gases; and carbon monoxide and methyl chloride as examples of non-irritant gases."

In order to demonstrate the many different ozone concentrations which have been reported to have or not to have physiological effects on humans or animals, a few of the many references available have been used to complete Table XIX. Each authors' designation as to effect and ozone concentration is listed.

TABLE XIX. PHYSIOLOGICAL EFFECTS OF OZONE

OZONE CONCENTRATION	EFFECT REPORTED	AUTHORS
1 ppm/vol	Nontoxic to school children	Bass (22) 1913
1 to 10 ppm	Death of dogs and rabbits	Jordon et al (165) 1913
15 to 20 ppm	Death of test animals, 2-3 hours	Schneckenberg (265) 1918
1.0 ppm	MAC*	Hill & Aeberly (146) 1921
0.5 ppm	MAC	Henderson & Haggard (137) 1936
0.01 ppm/vol	Detect by smell	Witheridge & Yaglou (320) 1939
0.01 ppm/vol	Irritating to nose and throat	"
0.04 ppm/vol	MAC	"
50 ppm	Nontoxic	Hill (145) 1942
1 ppm	MAC	Dalla Valle (56) 1945
1.0 ppm	MAC	Silverman (269) 1947
50 ppm/wt	Nontoxic in short periods	Thorp (287) 1950
20 ppm/wt	MAC	Thorp (287) 1950
4 ppm/wt	Nontoxic over long periods	Thorp (287) 1950
1.0 ppm	MAC	Ewell (82) 1951
0.1 ppm	MAC	Van Atta (291) 1951
0.1 ppm	MAC	American Medical Association (5) 1953

* MAC = Maximum allowable concentration.

One of the most common methods for the generation of ozone for medical or experimental purposes is the use of ozonizers. These machines use a "brush" discharge between high potential electrodes separated by air or oxygen. The output of the machine is influenced by the density of the current, the source of oxygen (air or pure oxygen) and the relative humidity (for air only). Air is generally used as the source of oxygen for air conditioning and general sanitation or for odor control purposes. Cylinders

of oxygen are usually employed by medical investigators. Ozonizers produce pure ozone only when pure oxygen is utilized (221). Conditions of high voltage, high humidity, and the use of air may result in the production of oxides of nitrogen, even in excess of the amount of ozone produced (284). The difference between the smell of pure ozone and ozone contaminated with oxides of nitrogen was described as the difference between clover or "new-mown hay" and chlorine or ammonia (286).

As early as 1913, Von Kupffer (293) and Czaplewski (54) mentioned the harmful effects of nitrogen oxides in ozone. However, supporting evidence was lacking until 1941 when Thorp (284) demonstrated how the toxicity was influenced by the ozone source and suggested that mixtures of nitrogen oxides and ozone were more toxic than ozone alone. Hill (145) in 1942 confirmed this supposition by using pure ozone and repeating experiments done in 1921 (146) with mixtures of ozone and oxide of nitrogen mixtures.

Thorp (285) reviewed the situation in 1950, evaluating the toxic limits given by various investigators on the basis of the purity of the ozone used, and came to the following conclusions:

(a) "..... ozone containing nitrogen oxides is toxic and exposure to concentrations of over 1.5 parts per million by weight for long periods of time may be considered definitely detrimental to humans."

(b) "Up to four parts per million (of pure ozone) may be classified as the 'non-symptomatic region', i.e., a worker exposed to concentrations in this range experiences no physical effects except odor. Several definite physiological changes, however, occur in this region; namely, a decreased BMR and lowered pulse rate."

(c) "Ozone concentrations as high as 50 ppm/wt for short periods of time have no irritating physical effect although there occurs a symptom termed, substernal pressure."

(d) "Twenty ppm/wt of ozone in air is non-toxic."

In regard to the poisonous nature of ozone, Hill (145) states, "pure ozone is not poisonous in any sense of the word as it breaks down in contact with the mucous membranes and only oxygen remains. For this reason, there are no cumulative effects and ozone may be breathed for long periods of time without harm, provided, of course, that the immediate irritations of strong concentrations is avoided."

Concentrations of ozone believed to be injurious to man were listed by Sollman (273) and Elford and Van de Ende (73) as follows:

CONCENTRATION ppm	EXPOSURE TIME	EFFECT
0.04	long time	safe
0.05	long time	irritation of throat
0.3-0.5	long time	respiratory irritation
1.0		affects O_2 use and CO_2 output
10	15 minutes	sore throat
5-10	short time	fatigue, drowsiness
5-10	long time	pulmonary edema, pneumonia

At the Armour Research Foundation the following LD_{50}'s were determined for animals during an exposure time of three hours:

Mice and rats	20 ppm ozone
Rabbits and cats	35 ppm ozone
Guinea pigs	50 ppm ozone

In the past, it was common practice to express the maximum allowable concentration for ozone as one ppm per volume (1.66 ppm/wt) in air, although some felt this amount was too high (291). In spite of the lack of agreement among workers in this field it appears certain that ozone, free from nitrogen oxides, is not as toxic as once supposed. Ewell (82) quoted several organizations who accepted this figure. The 1958 meeting of the American Conference of Government Industrial Hygienist set the threshold limit for ozone at 0.1 ppm per volume or 0.2 milligram per cubic meter of air (5).

Miller and Ehrlich (211) recently studied the susceptibility of mice and hamsters to respiratory infection with Klebsiella pneumoniae following exposure of the animals to one to four ppm ozone for various periods of time. Under the conditions of their tests pre-ozone treatment, in every instance, lowered the respiratory LD_{50} dose.

Recently, a number of animal studies have been made on the toxicity of ozone and on methods of therapy. While these will not be reviewed in detail, most substantiate the reasonableness of the present allowable concentration of 0.1 ppm.

E. PRODUCTION OF OZONE BY UV LAMPS

Some low pressure, mercury vapor, UV lamps produce a measurable amount of ozone. The type of glass used to make the tube determines the amount of UV radiation below 2000A which will be emitted and consequently the amount of ozone produced. UV lamps are usually designated as high, or negligible ozone producers. Sources of information giving precise amounts of ozone produced by UV lamps appear to be limited.

The amount of ozone produced by UV lamps decreases after the first 100 hours of use, after the glass has been seasoned. It has been shown (219) that if the concentration and decomposition coefficients (page 74) are known, the amount of ozone generated by a lamp can be calculated. Nagy gives the following example "..... if we assume the ozone concentration is 0.06 ppm/vol in a room 10 x 10 x 10 ft or 1000 cubic feet, the amount of ozone would be 0.00012 gram per cubic meter, or a total of 0.00012 x 28.23, or 0.00338 gram/1000 ft^3. Multiplying this by the decomposition coefficient we have:

$$0.23 \times 0.00338 = 0.000877 \text{ gm/min}$$

$$= 0.0526 \text{ gm/hr}$$

To calculate the ppm/volume, if we have the grams per hour:

$$\text{ppm/vol} = \frac{O_3 \text{ in grams} \times 1000}{2 \times 0.23 \times 60 \times M^3} \quad \text{n}$$

The average ozone output of several types of UV lamps has been determined using the method of Crabtree and Kemp (51) with the following results:

WL-794 - L UV Lamp

0.01 gm/hr
0.1 ppm/wt/220 ft^3
0.06 ppm/vol/220 ft^3

WL-782 - H - 30 UV Lamp (Cold cathode)

0.2 gm/hr
0.42 ppm/wt/1000 ft^3 = 0.25 ppm/vol
0.1 ppm/wt/4200 ft^3 = 0.06 ppm/vol

WL-782 - L - 30 UV Lamp (Cold cathode)

0.023 gm/hr
0.1 ppm/wt/500 ft^3
0.06 ppm/vol/500 ft^3

WL-782 - H - 10 UV Lamp

0.22 gm/hr
0.47 ppm/wt/1000 ft^3 = 0.28 ppm/vol
0.1 ppm/wt/4700 ft^3 = 0.06 ppm/vol

The above determinations were made in a 1780-cubic foot room with each lamp hung in the geometric center of the room without any enclosing fixture. Placing the lamps in fixtures will reduce the ozone by a factor of one-half to one-tenth. Proximity of a lamp to surfaces such as ceiling, duct walls, etc., will reduce the ozone output further. For these reasons, the above data cannot substitute for actual practical determinations if the amount of ozone present at a particular installation is desired.

F. GERMICIDAL ACTIVITY OF OZONE

1. Surface and Air-Borne Microorganisms

A review has been made of some of the articles and abstracts available dealing with the germicidal activity of ozone. There appears to be considerable disagreement among investigators concerning concentrations and exposure times necessary. This lack of agreement is probably the result of the use, in many cases, of impure ozone preparations, faulty ozone generators, or inadequate methods of measurement. These factors are discussed elsewhere. In this review an attempt has been made to select material where the action of specific amounts of ozone are given.

The ozone concentration germicidal for Achromobacter was found to be about 300 ppm per volume at 20°C and 10 to 100 ppm per volume at 0°C (111). Ewell (82), however, observed that at a given ozone concentration about the same lethal effect occurred at 4°C as at 20°C. Haines (112) indicated that Pseudomonas and Achromobacter were more resistant to ozone than Staphylococci, Proteus, Bacillus subtilis or Bacillus mesentericus. Bacteria suspended in water (1×10^6 cells per ml) were sterilized in two hours by 100 ppm per volume ozone. Haines (111) found that about equal amounts of ozone were required to inhibit the growth of molds and bacteria growing on agar plates. Between 10 and 1000 ppm per volume were required depending upon temperature, humidity, medium, and the age of the culture. The storage of eggs in three ppm per volume ozone in air markedly inhibited mold growth and caused no "off-flavors." In an atmosphere of high humidity, Ewell (82) reported that an ozone concentration of 0.6 ppm per volume in the air within egg cases would prevent the growth of molds on eggs. Mold growth on meat was prevented by maintaining about 1.5 ppm ozone in a storage-room atmosphere.

According to studies by Giese and Christensen (101), a concentration of 0.04 ppm ozone is adequate to inactivate aerosols of Streptococcus salivarious and Staphylococcus albus, although concentrations up to 330 ppm were required to kill Bacillus prodigiosus on a surface or in blood serum. Heiling and Scupin (134) stated that fungal spores were more susceptible than bacteria to the lethal action of ozone. Spores of four mold species, however, were found by Lea (182) to be capable of germination after exposure to 400 ppm of ozone for 10 to 15 hours, but were killed in 20 hours.

Ozone, at concentrations as low as 0.025 ppm, has been shown to be capable of killing organisms at a relative humidity of 60 to 90 per cent (73). Watson (298) mentioned a reduction of fungus spores and apple-rot disease by adding ozone to the air of an apple storage room. The growth of organisms on meat was found by Mallmann and Churchill (200) to be retarded by 0.1 ppm ozone. The control of mold upon cheddar cheese during ripening with 1.0 ppm ozone has been mentioned.

Experiments on the application of ozone for the sterilization of spun glass filter media have been conducted at Armour Research Foundation (15). When sections of 50 FG* spun glass were inoculated with up to 300 spores of Bacillus subtilis var. niger per square centimeter and exposed statically or dynamically to 0.02 or 5.5 per cent ozone, sterilization of the media did not occur at room temperature after exposures for as long as six hours. It was concluded that ozone did not have the penetrating properties for treatment of this type of filter media.

A review by Weaver, in 1951 (299), cited references which indicated that bacteria were difficult to destroy with ozone, even at high concentrations. Nagy (221) has criticised the inadequate techniques used by several workers cited in Weaver's references (165,264). Original experiments by Nagy gave the following results:

(a) The presence of ozone in a concentration of five ppm, in a duct of an air conditioning system, showed no bactericidal action because of the low humidity and short exposure time.

(b) Ozone concentrations from 0.05 to 0.1 ppm per weight in refrigerators showed germicidal action against Escherichia coli and Penicillium ilaticum on agar plates when exposure times from 24 to 64 hours were used.

(c) At 20°C and with an exposure time of 20 to 22 hours, from 90 to 100 per cent of the inoculum of E. coli on open Petri dishes were inactivated by 0.1 ppm per weight ozone.

There is no doubt that abundant moisture is essential for the bactericidal reaction of ozone. Also, there can be no doubt that ozone can inactivate microorganisms if sufficient concentrations are used for the correct exposure times. Unless these special conditions are established, traces of ozone probably exert very little germicidal power. Ozone produced by low pressure germicidal UV lamps designated as low ozone producing, probably will show no detectable germicidal effects because of the low penetration and short half-life of ozone.

Wheeler (316) installed ceiling germicidal lamps in Naval barracks and reported that very little ozone could be detected after the first 50 hours of use. Using a 30-watt germicidal lamp in a 2500-cubic foot room

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* American Air Filter Co., Louisville, Kentucky.

Luckiesh (185), in a series of studies on ozone production, found that the equilibrium concentration of ozone is less than 0.1 ppm if there is the slightest degree of ventilation. Koller (174) stated that the concentrations of ozone produced by germicidal lamps do not kill dry, air-borne organisms.

In biological installations, care should be taken that only low-ozone producing UV lamps are used.

2. Microorganisms Suspended in Liquid

Investigations of the germicidal action of ozone gas go back as far as 1892 when Ohlmuller (228) demonstrated the inactivation by this gas of the etiological agents of typhoid fever, cholera, and anthrax. Ohlmuller realized that organic material in water had an ozone demand to be satisfied before inactivation of organisms could proceed. In 1895 Van Ermengem (282) studied the rapid inactivation of several types of bacterial spores and E. coli as well as inactivation of a dilution of tetanus toxin by ozone. He also noted improvements in the color, taste, and odor of ozonized water. In 1899 Calmette et al (41) studied the killing of microbes in water by the action of ozone for the city of Lille, France. A variety of other references are available describing procedures for treating municipal water supplies in France and other European countries with ozone.

In this country Smith and Bodkin (270) evaluated the influence of pH on the germicidal action of chlorine and ozone. With residual ozone at a concentration of 0.13 to 0.2 ppm, a rise in pH necessitated a slightly increased exposure time to obtain sterility. Kessel et al (170) claimed that ozone was many times more effective than chlorine in inactivating poliomyelitis virus. Others have made similar studies (249). In 1944 Kessel et al (171) found that 0.3 ppm ozone residual in water was faster in its germicidal action than 0.5 to 1.0 ppm chlorine.

In liquids, ozone has been found to be an effective germicidal agent if used under controlled conditions. Water in the municipal systems of London, Berlin and Paris has been treated with ozone for many years, and, in this country, Hobart, Indiana; Whiting, Indiana; and Philadelphia, Pennsylvania use ozone-treated water (287). It has been stated that there are a total of 136 municipal water plants in which ozone is employed, serving approximately 8,000,000 people. Ozone is usually added to water by an air jet containing 2.5 to 5 grams of ozone per cubic meter of water. Contact time is usually 3 to 5 minutes. Pretreatment of the water is necessary. Although ozone does not introduce an odor or taste problem in potable water systems and is said to be more effective than chlorine against certain microorganisms, it lacks the residual effect typical in chlorinated waters. Although the effect of residual chlorine in case of pollution of a water system has been questioned from time to time, there has been no indication of wholesale acceptance of ozone as the preferred water sterilizing agent.

A recent study by Miller et al (212) showed that raw sewage highly contaminated with infectious microorganisms, including spores, botulinum toxin, and influenza virus could be effectively sterilized with ozone. Concentrations between 100 and 200 ppm were used. A total of 90 minutes exposure was required to obtain sterility, but a 99.9 per cent reduction was effected in five minutes. Most of the ozone could be recovered.

In 1954 Dickerman et al (64) showed that exposure of spore forming organisms in water to 1.0 ppm of ozone caused complete inactivation of the spores in five minutes. The initial inoculum was 70,000 organisms per milliliter of water. Raw stream water with low organic content was sterilized in five minutes with two ppm of ozone.

In Switzerland, in a modern soft drink factory, bottles are sterilized by introducing air containing 30 milligrams per liter of ozone for a period of 15 to 20 seconds (289).

It is clear that ozone is an excellent sterilizing agent for many liquids, including drinking water, swimming pool water, and sewage. Like any other disinfectant, its use requires a thorough understanding of its limitations and the proper methods of application. Although it has never been done on a practical scale, it is probable that ozone could be used to sterilize infectious effluents from infectious disease laboratories and hospitals.

3. Medical Uses

Many applications of ozone have been tried in medical and dental research, including the injection of ozone or ozone mixtures into the blood stream, fistulas, and muscles. In addition, various devices have been devised for exposing wounds and skin to ozone gas. Such references are included in the bibliography by Thorp (287).

G. DEODORIZING EFFECTS OF OZONE

A substantial amount of experimentation has been conducted to define the deodorizing effects of ozone gas. It is well established that ozone oxidizes many odor-producing compounds to produce less odoriferous substances. In some cases, however, ozone may produce a more obnoxious compound. For example, the reaction of ozone with formaldehyde produces formic acid which is not only more odoriferous but also more toxic (287). In some situations ozone may act only as a masking agent but, in general, it is believed to be an excellent deodorizing agent for many substances. Thorp (287) suggests that when ozone acts as a true deodorizer it should demonstrate this property at nontoxic concentrations.

Franklin (87) and Powell (242) gave results of tests on the oxidation of various food, tobacco, and putrefactive odors. Most gases emanating from these products were oxidized. Olsen and Ulrich (230) reported on the oxidation of ammonia and hydrogen sulfide, pointing out that oxidation of odors is a chemical reaction and is quantitative in the sense that a definite amount of ozone is necessary to oxidize a definite quantity of oxidizable material. This point was also discussed by McCord and Witheridge (204). Kupper (177) reported on the oxidation of tobacco smoke and odors in hospitals and public buildings, using as low as 0.05 ppm ozone. Hill (145) made tests with cigarette and cigar smoke as well as hydrogen sulfide, perfumes, urine, butyric, and acrylic acid and found that all of these odors can be oxidized by low ozone concentrations if sufficient time is allowed.

Czaplewski (55) stated that some odors are oxidized while others are weakened. Feldner (83) showed that ozone eliminated odors in hospital rooms containing patients being treated for gastroenteritis. Bordas (33) found that odors formed as a result of fermentation of organic matter were destroyed by ozone. Boyer (35) reported that the underground railroad tunnel in Paris was successfully deodorized by ozone. Other observations have been made on the removal of odors by ozone in air circulating systems, e.g., the reports of Anderegg (8), Hallett (114), Franklin (87), Woodridge (324), and Vosmaer (295). Witheridge and Yaglou (320) found that as low as 0.015 ppm ozone was effective in reducing odors.

Ozone has been used in cold storage rooms to destroy odors from meats, fruit, vegetables, and putrefaction. The odors from fruit, probably ethylene, are destroyed as shown by the retardation of ripening. Ewell (77,79, 80,81) conducted experiments on the use of ozone in cold storage rooms and summarized the work of others on the subject. Hartman (131) also has successfully used ozone for the oxidation of fruit and vegetable odors in cold storage compartments.

There are some opinions that ozone only acts as a masking agent. As an example, Erlansden and Schwartz (76) are cited as having found ozone to have no effect on tobacco smoke, indole, skatole, ammonia, hydrogen sulfide, and other odoriferous compounds except as a masking agent. Sawyer et al (264) also state that ozone masks odors.

Nagy (220) conducted a series of laboratory tests with ozone at a concentration of approximately 0.1 ppm per weight. Acrolein, an aldehyde having the odor of burning fat, was converted to a nonodoriferous compound in 10 to 16 hours. Allyl sulfide, methyl thiocyanate, indole, and skatole were also rendered nonodoriferous by exposure to ozone. However, normal propyl disulfide, a compound which does not contain double bonds and therefore is not converted to an ozonide, was not deodorized by exposure to ozone. It was concluded that compounds with unsaturated bonds were the most readily oxidized and that the amount of ozone must be in stoichiometric proportions.

VIII. FACTORS AFFECTING THE BIOLOGICAL ACTION OF UV RADIATION

In a report such as this on the practical use of germicidal radiations in infectious disease laboratories, it is not necessary to present detailed discussions of the various related factors which have been the subject of vast numbers of laboratory experiments. These studies, both past and present, have been adequately summarized (149) and are, in general, less pertinent to the problem of practical uses for UV radiation than the basic studies on genetics, cytochemistry, etc. Therefore, in this chapter, only a limited discussion of some basic factors affecting the action of germicidal UV radiation will be discussed. The reader is urged to consult other works for more complete discussions.

A. TEMPERATURE

Gates (94) determined the temperature coefficient of the bactericidal reaction of UV radiation at 2540A. Using temperatures of 36°, 21°, and 5°C and exposing inoculated agar plates, he plotted the progressive rate of killing and also calculated the average temperature coefficient of the bactericidal reaction for 100 per cent inactivation of Staphylococcus aureus. The temperature coefficient was 1.06. From this, he concluded that the reaction is physical (or purely photochemical) rather than chemical.

According to Luckiesh (185) the temperature of the air does not appear to affect the resistivity of microorganisms for the range of temperature commonly encountered in interiors and in ventilation systems.

B. pH

A series of parallel experiments (94) indicated that slight changes in the susceptibility of S. aureus to UV radiation occurred when the pH of the suspending medium was varied from 4.5 to 7.5. At pH 9.0 and 10.0 there was an increase in the susceptibility of the test organism. These results are shown in Figure 23.

Other workers have shown that when acid media were used, the bactericidal action was greatly accelerated. Bacteria in medium of pH 6 to 8 were about ten times as resistant as when suspended in medium of pH 2.0.

C. AGE OF CULTURE

Gates (94), working with S. aureus, tested the differences in susceptibility of 4-, 28-, and 52-hour old cultures to UV radiation and found that resistance increases with the age of the culture.

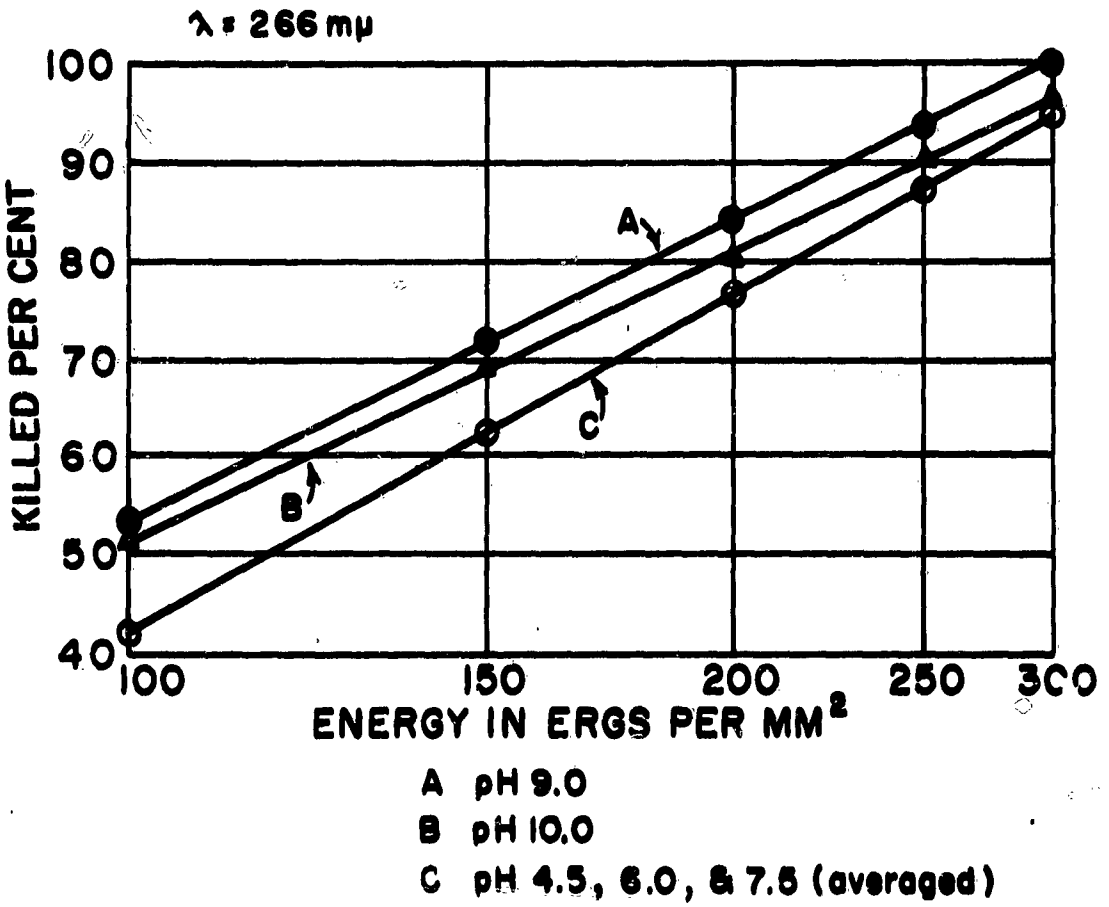

Figure 23. Per Cent Bacteria Killed at Various Media Hydrogen Ion Concentrations. Gates (94)

The susceptibility of organisms in different growth phases has been reported by other workers (60,321,325). It is generally agreed that strains of Escherichia coli are more resistant to UV radiation when in the stationary growth phase.

Studies by Giese et al (102) with Saccharomyces cerevisiae show this organism to be more resistant to UV radiation when in the logarithmic phase of growth than when just past the logarithmic phase. Nitrogen starvation increased yeast susceptibility to 2537A radiation. Nitrogen starved cells became more resistant to radiation inactivation when organic but not when inorganic nitrogen was supplied. As in other tests, the presence or absence of oxygen made little difference in ultraviolet sensitivity.

The most recent studies of Romig and Wyss (257) have helped to explain the differences which have been reported in the susceptibility to UV radiation between aerobic vegetative bacilli and their spores. By using the endotrophic sporulation technique of Hardwick and Foster (117), these investigators showed that radiation resistance develops before the appearance of the mature spores and, in fact, is manifest before the development of heat resistance. In slowly sporulating cultures of Bacillus cereus the prespore stage (forespore) exhibited increased resistance to radiation treatment and became less susceptible to photoreactivation, whereas, resistance to heat treatment at 65°C for 15 minutes was not maximal until two hours later when mature spores predominated. Thus, the exact state of the spores may determine their relative resistance to UV irradiation. This theory is further supported by studies which have shown that when spores are placed in an environment suitable for germination, sensitivity to UV destruction rapidly returns (208,277).

D. RELATIVE HUMIDITY

1. Literature Review

Luckiesh (185) and other workers (173,305,306) have published effects of relative humidity on the germicidal effectiveness of UV radiation. Luckiesh concludes that the resistivity of E. coli in air at a relative humidity of 75 per cent is twice that for a relative humidity of 35 per cent. Wells (306) and Whisler (318) claim that the bactericidal effectiveness of UV radiation on air-borne E. coli decreases greatly as relative humidities rise above 60 per cent. Their results are similar to those of Gates (94) shown in Figure 24.

The view that relative humidity affects the bactericidal reaction is not supported by other authors (245,246,247). Nagy (220) states that the energy necessary to inactivate a microorganism is the same regardless of the moisture conditions and points out the following:

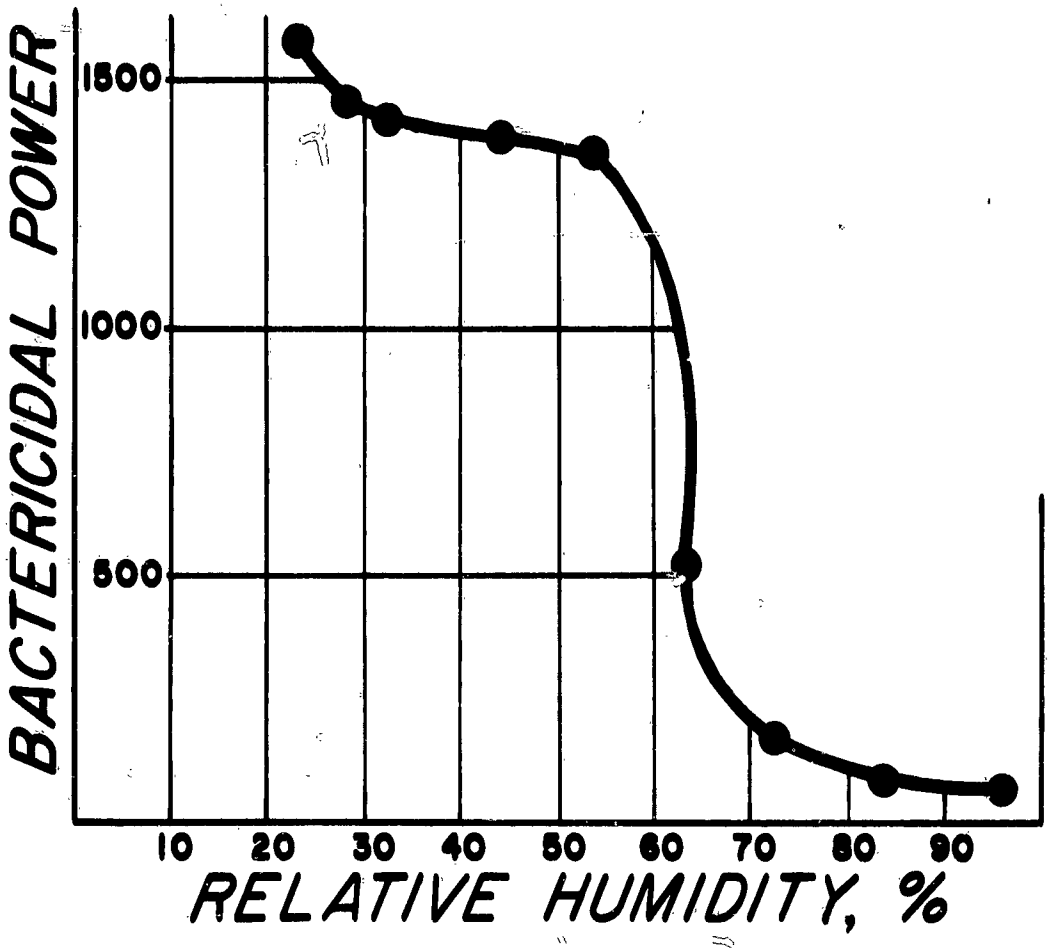

Figure 24. Effect of Relative Humidity on the Bactericidal Action of UV Radiation. Gates (94)

(a) The sampling devices used by Whisler and Wells were selective and did not give a true representation of the bacterial population.

The probe used by these authors sampled only the small particles at high air velocities while the centrifuge removed only the largest particles collected by the probe. Relative humidity would affect the ratio of the particle sizes and thus the proportion of organisms sampled. At high humidity and low air velocity a large number of organisms would be collected by this method, indicating poor UV efficiency. At high air velocity and low humidity the organisms would not be collected, which was interpreted as high UV efficiency.

When these factors were controlled, the amount of energy required to destroy an organism in air at low humidity was the same as for a similar organism on a Petri plate.

(b) The greater apparent efficiency of UV radiation in air ducts can be correlated with the Reynolds number which is a measure of air turbulence. Particles in turbulent air receive more energy than similar particles in linear air flow.

A recent study by Beebe and Pirsch (24) on the effect of simulated sunlight radiation on air-borne Pasteurella pestis and Pasteurella tularensis showed a radiation protection effect with high humidities. The authors suggested that the protective effect associated with the moisture content of the air might be explained by the presence of dissolved solids in the moisture surrounding the cell. This moisture layer would not be more than one micron in thickness. However, many other factors such as air temperature, visible light, infrared radiation and particle temperature may be involved.

There appears adequate justification for considering the relative humidity in practical UV applications. Whisler (318) points out that the effect may be physical rather than biological in nature. At lower humidities it seems reasonable to suppose that there will be smaller particles, and less clumping and shielding, therefore, a greater percentage of the exposed organisms can be "hit."

2. Experimental

In view of the conflicting reports on the effect of humidity on the bactericidal effect of UV, an experiment was conducted to determine the effect of relative humidity on the survival of Bacillus subtilis var. niger spores exposed to UV radiation.

a. Methods

An aerosol of B. subtilis spores was passed through an aluminum tube five inches in diameter containing one G36T6 UV lamp. The relative humidity of the aerosol air mixture was regulated by controlling the secondary (mixing) air supply. This was done by passing the air through water

for adding moisture or through silica gel for removing moisture. The controlled aerosol was passed through a mixing chamber, where large particles settled out, and then past wet and dry bulb thermometers for RH determinations. Air emerging from the exposure chamber was sampled with sieve type air samplers, using corn steep agar plates. A schematic diagram of the apparatus is shown in Figure 25.

Air flow through the UV chamber was regulated to one cubic foot per minute. The UV tube was partially covered with tape to reduce the germicidal action and allow survival of a percentage of the spores.

At each relative humidity tested, air samples were taken with the UV lamp off and with it on. From these data the per cent penetration of the aerosol at that relative humidity was calculated.

b. Results

The results of 3 tests - each including 8 trials at different humidities - are tabulated in Tables XX, XXI, and XXII. These results are also shown in graphic form in Figure 26. It can be seen that the per cent recovery of irradiated spores varied only slightly from trial to trial with no apparent relationship to the relative humidity. For bacterial spores, under the conditions of this experiment, large changes in the moisture content of the aerosol-air mixture had little or no effect on the germicidal effectiveness of the radiation.

E. IRRADIATION OF MEDIA

Coblentz and Fulton (46) exposed open sterile agar plates to UV radiations of wave lengths 2100A to 2960A. They found no detrimental effects on the subsequent ability of the medium to support growth except where very high concentrations of radiation were used. In studies of this type by the authors, four per cent blood agar, nutrient agar and eosin methylene blue agar plates were exposed to a radiation intensity of 85 microwatts per square centimeter for varying lengths of time up to a maximum time of four hours. Irradiated plates then were inoculated with 0.1 milliliter of a diluted suspension of the test organisms: S. marcescens, B. subtilis var. niger, and E. coli. Nonirradiated agar plates were used for the controls. After incubation, the counts on the exposed and nonexposed plates were compared. Even after four hours of irradiation, there was no change in the ability of the agar plates to support growth. Therefore, it was concluded that exposure of agar surfaces to UV radiation doses as high as 20,400 microwatt minutes per square centimeter has no effect on subsequent inoculation and growth of S. marcescens, B. subtilis, or E. coli. Open Petri dishes containing sterile agar are often exposed in an UV chamber (e.g. a walk-in incubator) in order to dry the agar surfaces.

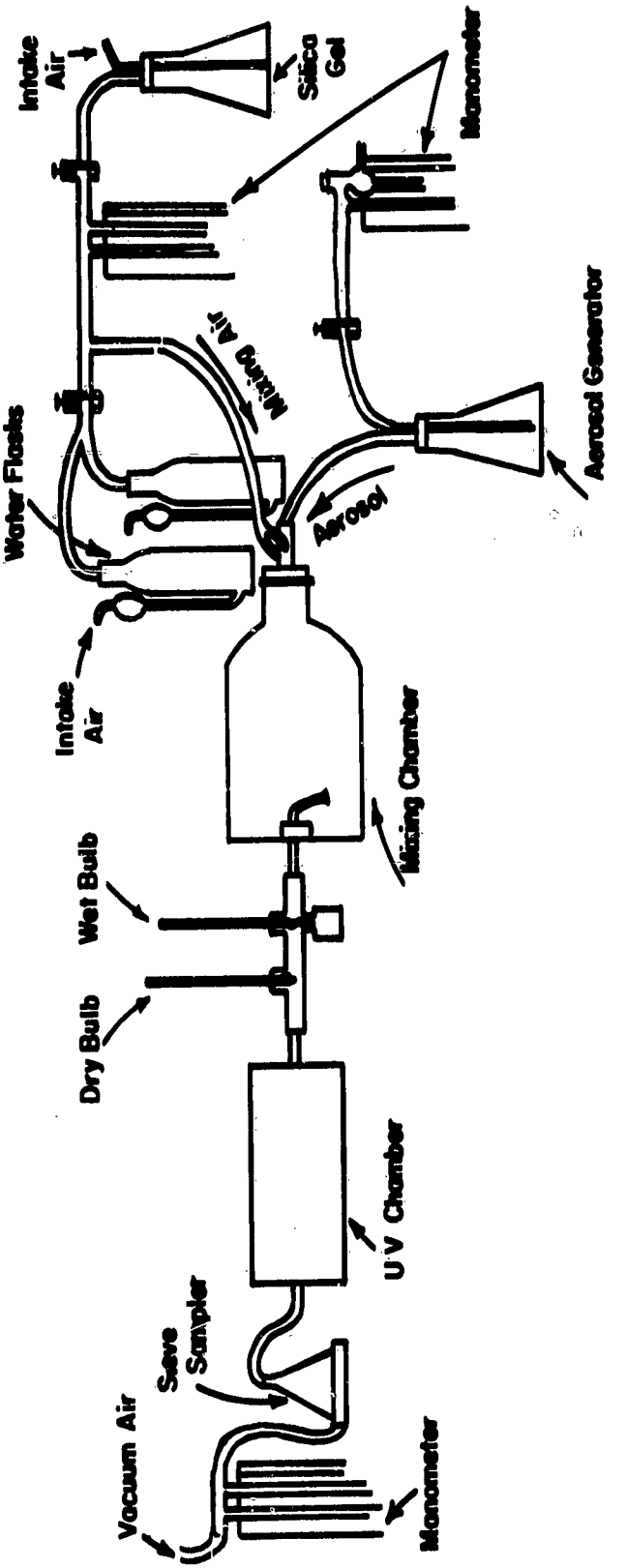

Figure 25. UV Humidity Apparatus.

TABLE XX. SURVIVAL OF UV IRRADIATED B. SUBTILIS SPORES AT VARIOUS RELATIVE HUMIDITIES[a]
(Test 1)

RELATIVE HUMIDITY, per cent	TEST ORGANISMS RECOVERED			PER CENT PENETRATION
	UV Off	UV On		
	org per cu ft	org per cu ft	avg per cu ft	
29	1.4×10^2	2.6 3.6 3.4	3.3	2.36
37	3.2×10^2	6.4 7.2 7.4	7.0	2.5
43	2.2×10^2	7.4 6.0 4.6	6.0	2.73
50	3.3×10^2	7.2 8.8 10.0	8.4	2.55
66	3.6×10^2	8.4 9.2 7.8	8.5	2.36
72	4.2×10^3	9.6 10.6 8.2	9.5	2.26
86	3.1×10^3	6.6 5.8 5.2	5.9	1.90

a. The radiation intensity was the same at all RH's.

TABLE XXI. SURVIVAL OF UV IRRADIATED B. SUBTILIS SPORES AT VARIOUS RELATIVE HUMIDITIES[a]
(Test 2)

RELATIVE HUMIDITY, per cent	TEST ORGANISMS RECOVERED			PER CENT PENETRATION
	UV Off	UV On		
	org per cu ft	org per cu ft	avg per cu ft	
33	1.24×10^2	3.0 4.0 3.6	3.52	2.85
40	1.29×10^2	3.6 3.2 4.6	3.8	2.93
48	1.45×10^2	5.6 4.0 5.8	5.13	3.53
55	1.71×10^2	6.4 5.8 5.6	5.93	3.47
62	1.69×10^2	5.4 6.6 6.4	6.13	3.62
68	1.61×10^2	7.8 7.2 7.6	7.52	4.66
76	1.67×10^2	7.6 8.0 7.2	7.60	4.54
91	1.44×10^2	5.8 4.0 4.8	4.86	3.37

a. The radiation intensity was the same at all RH's.

TABLE XXII. SURVIVAL OF UV IRRADIATED B. SUBTILIS SPORES AT VARIOUS RELATIVE HUMIDITIES[a]
(Test 3)

RELATIVE HUMIDITY, per cent	TEST ORGANISMS RECOVERED			PER CENT PENETRATION
	UV Off	UV On		
	org per cu ft	org per cu ft	avg per cu ft	
29	1.24×10^2	3.8 3.2 4.4	3.8	3.06
36	$.89 \times 10^2$	1.8 1.2 4.0	2.3	2.56
41	1.33×10^2	2.6 2.4 3.0	2.7	2.10
48	1.26×10^2	3.4 2.2 3.0	2.7	2.14
54	$.76 \times 10^2$	2.4 1.6 2.2	2.1	2.76
63	1.35×10^2	5.0 5.6 6.4	5.66	4.16
70	1.23×10^2	4.0 4.4 5.0	4.43	3.60
88	1.25×10^2	4.4 4.6 5.0	4.66	3.72

a. The radiation intensity was the same at all RH's.

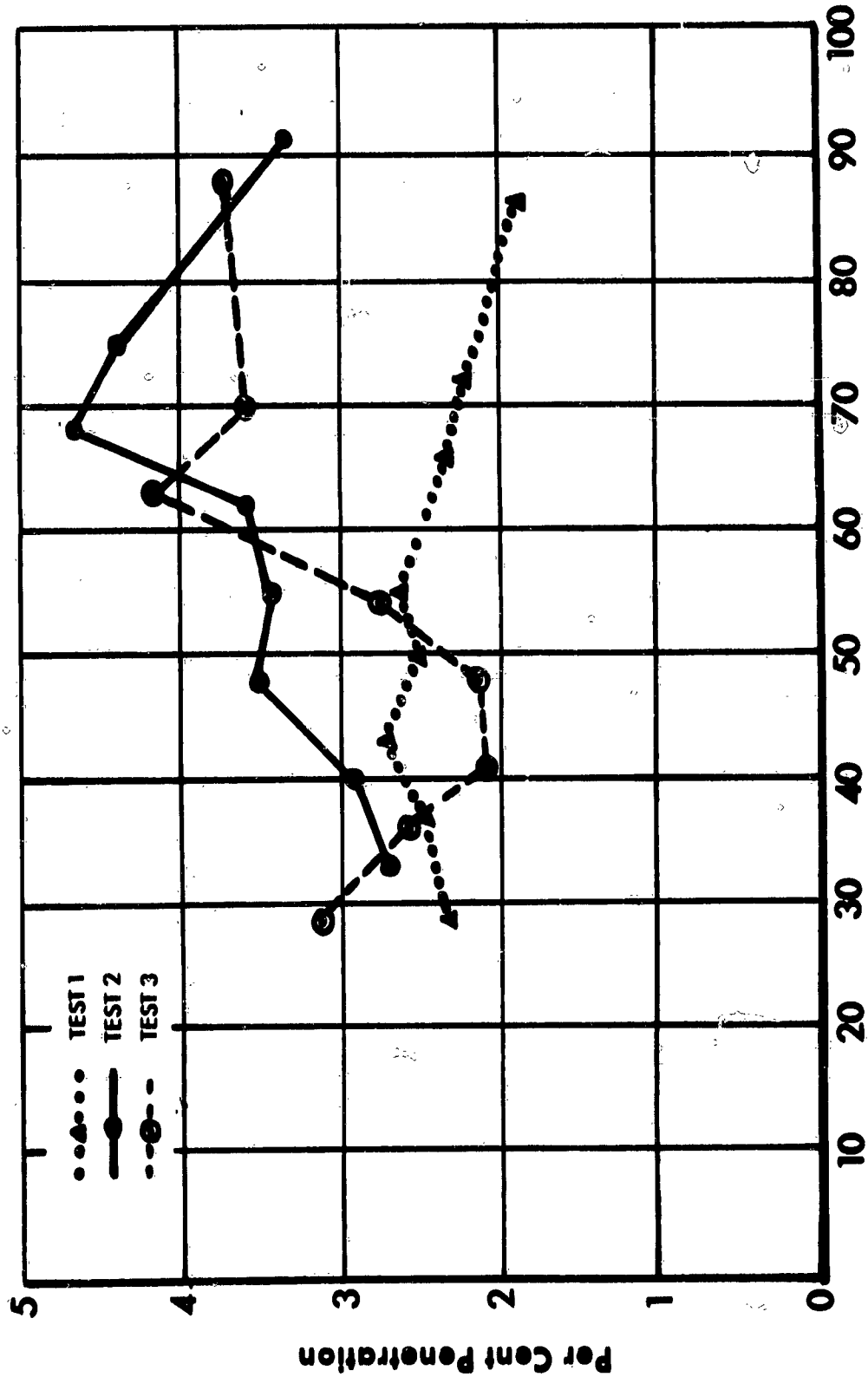

Figure 26. Per Cent Survival of UV Irradiated B. subtilis Spores at Varying Relative Humidities.

Experiments conducted at the University of Michigan by Laurence and Graikoski (180) indicate, however, that certain liquid media may be adversely affected by UV irradiation to the extent that subsequent inoculation gives lower yields of total cells. A chemically defined medium (Difco Niacin Assay Medium) was irradiated (wave length and intensity not given) for various lengths of time and then inoculated with Lactobacillus arabinosus. Growth after incubation was measured turbidimetrically. The results showed a linear decrease in growth with the time of irradiation. The observed effect was not the result of a change in pH. Attempts to reconstitute irradiated media by adding certain ingredients after irradiation failed to give growth comparable to that of the unirradiated control. All of the components of the medium used in these tests were essential for growth of the test organism. It was postulated that the formation by UV irradiation of toxic products, such as organic peroxides, may have been responsible for the deleterious effect.

F. HEAT SENSITIVITY

Cells which have been exposed to sublethal doses of UV radiation are more sensitive to heat than untreated cells. The experimental evidence has been summarized in a review by Giese (100). This phenomenon has been noted on work with protein solutions, bacteria, yeasts, and tobacco mosaic virus. The thermal death of irradiated bacteria occurs at a lower temperature or upon a shorter exposure than unirradiated cells of the same strain. This is not a mere additive effect because, if heat is applied preceding the radiation exposure, no difference in the UV susceptibility of the cells is noted. Rentschler and Nagy (246) show the same phenomenon and state that it is "incompatible with 'single photon hit' theory."

G. PHOTOREACTIVATION

Under certain conditions, it has been found that cells made nonviable by exposure to UV radiation can be reactivated by exposure to visible light. This was first demonstrated by Kelner, who worked first with Streptomyces griseus (167) and later with Escherichia coli (168). A similar phenomenon has been shown to occur with yeasts, bacteriophage, sea urchin eggs, and protozoa (100). In the technique usually employed, water suspensions of organisms are exposed to UV radiation doses sufficient to prevent subsequent multiplication of approximately 90 per cent of the cells. After exposure, duplicate cultures are held in the dark and exposed to a strong intensity of visible light or long wave length ultraviolet. The capacity for photoreactivation disappears after storage in the dark for two to three hours.

Kelner's discovery has led to several new theories regarding the mechanism of UV action. Most of the theories involve the formation of toxic substances. It has been postulated, for instance, that when bacteria are irradiated with UV radiation, a poisonous substance is produced in two

forms, one light labile and one light stable (100). Bacterial spores have been reported not to be subject to photoreactivation following treatment with UV radiation (257).

One complicating factor of the photoreactivation phenomenon is the fact that some laboratory strains of microorganisms are sensitive to the photoreversing wave length used (147,232,257,276).

Although photoreactivation is easily demonstrated under controlled laboratory conditions, it is questionable that it has any real application in the practical use of UV radiation where generous dosages of germicidal energy are applied in the presence of visible light.

Recent investigations on reactivation involve the use of various metabolites to restore cells inactivated with UV radiation. The work of Heinmets, et al (136), for example, suggests that cellular death by UV radiation is not a single step process and that a series of changes may occur depending upon the UV dose. Incubation of irradiated cells for about 17 hours with metabolites from the citric acid cycle causes an increase in the viable cell count, a phenomenon called "metabolic reactivation."

The chemical reactivation reported by Heinmets, et al (135) has been questioned by several other workers (92,158). The objections were based on tests which showed that it was almost impossible to remove all traces of nitrogen from the solutions in which reactivation takes place. Thus, multiplication of small numbers of surviving organisms during incubation in the metabolite might be confused with reactivation. Hurwitz, et al (158) performed experiments the results of which support this thesis. The recent extensive review by Jagger (161) is recommended for those interested in photoreactivation.

IX. GERMICIDAL EFFECTS OF UV RADIATION

A. GENERAL

Of all UV phenomena, the lethal action of radiant energy against microorganisms is probably the most frequently investigated. The voluminous information available usually can be classified under one of the following headings, in terms of the wave length and UV source used:

(a) Investigations using sunlight as the radiation source,

(b) investigations using the entire radiation from a source such as a carbon arc lamp or a high-pressure mercury lamp,

(c) investigations using arc lamps and crude filters such as Petri dish covers or window glass,

(d) investigations using selective filters or prisms which isolate fairly narrow regions of the spectrum, and

(e) investigations using low pressure, mercury vapor lamps, which emit about 95 per cent of their radiations in the 2537A wave-length band.

When summarising the quantitative effects of monochromatic UV radiation on microorganisms, only data under (c) and (d) can be considered. Even then, other limitations have been found that prevent accurate comparison of data obtained by various investigators.

Some of these limitations are:

(a) Differences in exposure techniques,

(b) differences in concentration of exposed microorganisms, and

(c) differences in determining and expressing the intensity of the UV radiation.

This last limitation is encountered most frequently. In some reports the only indication of the intensity is the distance of the exposed material from the UV source. Other studies have simply ignored the UV intensity. Ultraviolet readings have been made in terms of energy (ergs), power (watts or microwatts) or in terms of radiant power per unit area per unit of time (microwatt-minutes per square centimeter). Also, other units have been employed which represented a certain percentage destruction of a standard test strain under standard conditions. These terms are discussed in detail in the chapter on measurement. The terms most commonly used today to express dose of ultraviolet per unit area are microwatt-minutes per square centimeter and microwatt-seconds per square centimeter.

Although an enormous amount of experimental evidence has accumulated which proves that microorganisms are susceptible to the lethal action of UV radiation, the limitations listed above apply to much of this work. The following species or genera have been listed by various authors (75, 151,174,183) as susceptible to UV radiation.

African horse-sickness virus
Alcaligenes
Amoeba
Bacillus anthracis
Bacterium paratyphosa
Bacillus subtilis
Bacterium typhosa
Bacterium coli bacteriophage
Bacteriophage dysentery
Brucella
Cheese molds
Chicken pox virus
Cholera bacteriophage
Coliforms
Corynebacterium
Diplococcus pneumoniae
Dysentery bacteriophage
Eberthella typhosa
Encephalitis virus
Encephalomyelitis virus
Foot-and-mouth disease virus
Hemophilus
Herpes virus
Influenza virus
Klebsiella
Lactobacillus
Measles virus
Micrococcus
Mumps virus
Mycobacterium tuberculosis
Neisseria
Paramecium
Poliomyelitis virus
Proteus
Saccharomyces ellipoideus
Salmonella
Serratia
Shigella
Staphylococci
Staphylococci bacteriophage
Streptococcus
Tobacco mosaic
Tomato bushy stunt virus
Thermophilic sugar bacteria
Vaccinia
Vibrio

B. EFFECTS ON BACTERIA

1. Literature Review

In studies on the effects of UV radiation on bacteria, three methods of exposure are generally used: the agar plate method, the liquid suspension method, and the air suspension method. The agar plate method has been widely used and the results obtained can more easily be compared with other data of the same type. The data in Table XXIII are taken from a summary table in a review by Hollaender (149). The individual references are not shown, but may be found in the original review. Table XXIV shows similar information supplied by Westinghouse Electric Corporation. Variations found in the reported quantitative effects of UV radiation (2537A) on various strains of bacteria are vividly demonstrated in Table XXIV. For example, it is not likely that E. coli cells are more resistant to UV radiation than are the spores of Bacillus megaterium. Undoubtedly, variables such as pigmentation, cell concentration, and physical state of the culture are responsible for variances. The phenomenon of the shielding of one cell by another must not be overlooked. Many observations have indicated this to be important in the exposure of bacterial cells on agar surfaces.

The amount of energy necessary to destroy a bacterium has been studied by Hollaender and Claus (152). The authors reported that 13.1 x 10^{-6} ergs per bacterium are necessary to inactivate 36.8 per cent of the cells in a 15-hour culture of E. coli. Only 6.1 x 10^{-6} ergs per bacterium were necessary if the cells were first washed. In another paper on the effect of radiation on nematode eggs (154), approximately one erg at 2640A per egg produced a 50 per cent inactivation. Mold spores of Trichophyton mentagrophytes were destroyed by Hollaender (149) by exposure to 7 x 10^{-4} to 18.5 x 10^{-4} ergs per spore.

Based on an assumed molecular weight, Spector (274) gives the following values for T-1 and T-2 phages which resulted in loss of activity against the host cell, E. coli B.

PHAGE	WAVE LENGTH	QUANTUM EFFICIENCY (Number of molecules reacting per number of quanta absorbed)
T-1	2220A	5.0 x 10^{-4}
T-1	2537A	6.3 x 10^{-4}
T-1	3022A	7.3 x 10^{-4}
T-2	2220A	2.7 x 10^{-4}
T-2	2537A	3.1 x 10^{-4}
T-2	3022A	1.8 x 10^{-4}

TABLE XXIII. MICROWATT-MINUTES PER SQUARE CENTIMETER (ET VALUE) NECESSARY TO REDUCE COLONY FORMATION 90 PER CENT ON AGAR PLATES (Hollaender, 143)

ORGANISM	ET VALUE FOR 90 PER CENT KILL
Bacillus anthracis	75.3
B. megaterium sp. (veg)	13.8
B. megaterium sp. (spores)	45.5
B. paratyphosus (avg of 3 strains)	53.3
B. subtilis (mixed)	116.3
B. subtilis (spores)	100.0
	200.0
Corynebacterium diphtheriae	56.1
Eberthella typhosa	35.6
Escherichia coli	50.0
Micrococcus candidus	100.8
Micrococcus piltonesis	135.0
Micrococcus sphaeroides	166.6
Neisseria catarrhalis	73.3
Phytomonas tumefaciens	73.3
Proteus vulgaris	44.0
Pseudomonas aeruginosa	91.6
Pseudomonas fluorescens	58.3
Salmonella enteritidis	66.6
Salmonella typhimurium (avg of 3 strains)	133.3
Sarcina lutea	328.3
Serratia marcescens	40.3
	36.6
	13.8
Dysentery bacilli (avg of 5 strains)	36.6
Shigella paradysenteriae	26.0
Spirillum rubrum	73.3
Staphylococcus albus	30.6
	55.0
	30.6
	36.3
Staphylococcus albus	43.3
	82.5
Streptococcus hemolyticus	36.0
Streptococcus lactis	102.5
Streptococcus viridans	33.5

TABLE XXIV. INCIDENT ENERGIES AT 2537A MILLIMICRONS NECESSARY TO INHIBIT COLONY FORMATION IN 90 AND 100 PER CENT OF THE TEST ORGANISMS*

Note: Divide each number by 60 to obtain microwatt-min/sq cm (ET).

ORGANISM		ENERGY (MW-sec/sq cm)	
		90 per cent	100 per cent
BACTERIA			
Bacillus anthracis		4250	8700
S. enteritidis		4000	7600
B. megaterium sp. (veg)		1300	2500
B. megaterium sp. (spores)		2730	5200
B. paratyphosus		3200	6100
B. subtilis		5800	11000
B. subtilis spores		11600	22000
Corynebacterium diphtheria		3370	6500
Eberthella typhosa		2140	4100
Escherichia coli		3000	6600
Micrococcus candidus		6050	12300
Micrococcus sphaeroides		10000	15400
Neisseria catarrhalis		4400	8500
Phytomonas tumefaciens		4400	8500
Proteus vulgaris		3000	6600
Pseudomonas aeruginosa		5500	10500
Pseudomonas fluorescens		3500	6600
S. typhimurium		8000	15200
Sarcina lutea		19700	26400
Serratia marcescens		2420	6160
Dysentery bacilli		2200	4200
Shigella paradysenteriae		1680	3400
Spirillum rubrum		4400	6160
Staphylococcus albus		1840	5720
Staphylococcus aureus		2600	6600
Streptococcus hemolyticus		2160	5500
Streptococcus lactis		0150	8800
Streptococcus viridans		2000	3800
YEAST			
Saccharomyces ellipsoideus		6000	13200
Saccharomyces sp.		8000	17600
Saccharomyces cerevisiae		6000	13200
Brewer's yeast		3300	6600
Baker's yeast		3800	8800
Common yeast cake		6000	13200
MOLD SPORES	Color		
Penicillium roqueforti	green	13000	26400
Penicillium expansum	olive	13000	22000
Penicillium digitatum	olive	44000	88000
Aspergillus glaucus	bluish green	44000	88000
Aspergillus flavus	yellowish gr	60000	99000
Aspergillus niger	black	132000	330000
Rhizopus nigricans	black	111000	220000
Mucor racemosus A	white gray	17000	35200
Mucor racemosus B	white	5000	11000
Oospora lactis	white	5000	11000

* Supplied by Westinghouse Electric Corporation.

In addition, Spector (274) has collected data on the LD_{50} radiation dose for various unicellular organisms. For wave length 254 millimicrons (mμ), the LD_{50} values were as follows:

ORGANISM	LD_{50} DOSE, ergs/sq mm (254 mμ)
BACTERIA	
Escherichia coli	44-208
Micrococcus candicans	341
Pseudomonas pyocyanea	291
Staphylococcus aureus	66-275
Serratia marcescens	57
FUNGI	
Saccharomyces cerevisiae (dark)	503-900
Saccharomyces cerevisiae (light)	1400
Neurospora crassa, microconidia (dark)	370
Neurospora crassa, microconidia (light)	1020
Neurospora crassa, macroconidia (dark)	1440
Neurospora crassa, macroconidia (light)	3000
PROTOZOA	
Amoeba proteus (dark)	2160

For the practical application of UV radiation as a bactericidal agent, such information is not necessary. Data showing the intensities and exposure times required to inactivate test organisms under a variety of test conditions are applicable because variances due to shielding, cell concentration, and physical state of the culture are already included. If such information is accurate, practical installations employing radiant energy can be designed in accordance with the intensity requirements with an assurance that the desired results will be obtained.

Luckiesh (185) presented a graph showing intensities and exposure times required to give various ratios for bacteria and molds. This graph has been duplicated in Figure 27. Such data are difficult to apply in practice, and are subject to several criticisms. No mention is made of the number or different species of microorganisms involved. There is an obvious discrepancy in the line for "*B. coli*" in water and *B. subtilis* spores, as the latter organism is definitely many times more resistant to UV radiation than the vegetative *E. coli* organism. The intracellular nature of viruses would suggest that their inactivation in practical instances might be difficult. In addition it is doubtful that all members of any biological population show equal susceptibility to UV radiation. Extrapolation of radiation data is not a good practice.

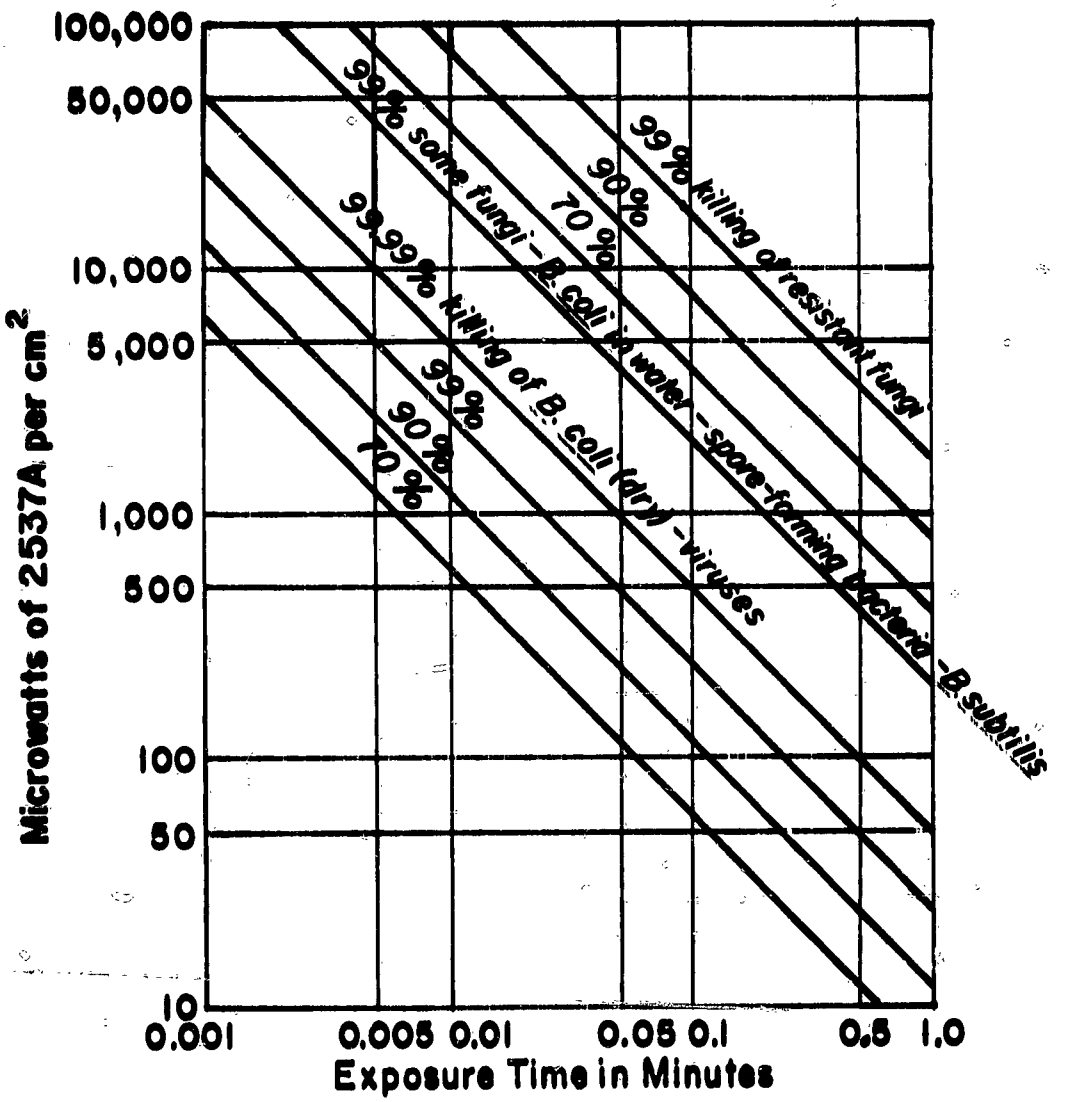

Figure 27. UV Intensity and Exposure Time Required to Give Various Values of Survival Ratios for Bacteria and Molds. Luckiesh (185)

2. Experimental

a. In Water Suspensions

Thirty-watt, hot cathode lamps were used in these experiments. Test bacteria were grown on agar slants for 24 hours and washed twice in sterile distilled water. Then, 20 milliliters of the suspension were placed in an open Petri dish. The depth of the bacterial suspension in the Petri dish was approximately 0.5 centimeter. Control counts were made of the suspension and the Petri dishes were then placed below the UV lamp at various distances for varying periods of time. After exposure, counts were made to determine the number of viable organisms remaining, and results were recorded as per cent survival or per cent kill.

An UV intensity meter was used to make measurements at the spots where the plates were exposed. These measurements gave the intensities of radiant energy in microwatts per square centimeter. This value, multiplied by the minutes of exposure gave an ET (intensity x time) value. As seen in Table XXV, when the ET values varied from 7 to 850, the per cent inactivation of Serratia marcescens cells in water varied from 0 to 99.9999. Each value is an average of at least three exposures. From these values, the survival curve shown in Figure 28 was made.

TABLE XXV. SURVIVAL OF WASHED S. MARCESCENS CELLS IN DISTILLED WATER EXPOSED TO RADIATION FROM A 30-WATT HOT CATHODE LAMP

ET MICROWATTS PER SQ CM X MINUTES	PER CENT KILL
7	0
9	40
12.5	45
15	50
18	68.2
25	72.3
45	77.7
62	94.75
72	98.27
75	98.59
100	99.62
125	99.905
170	99.989
250	99.991
255	99.991
340	99.995
425	99.999
850	99.9999

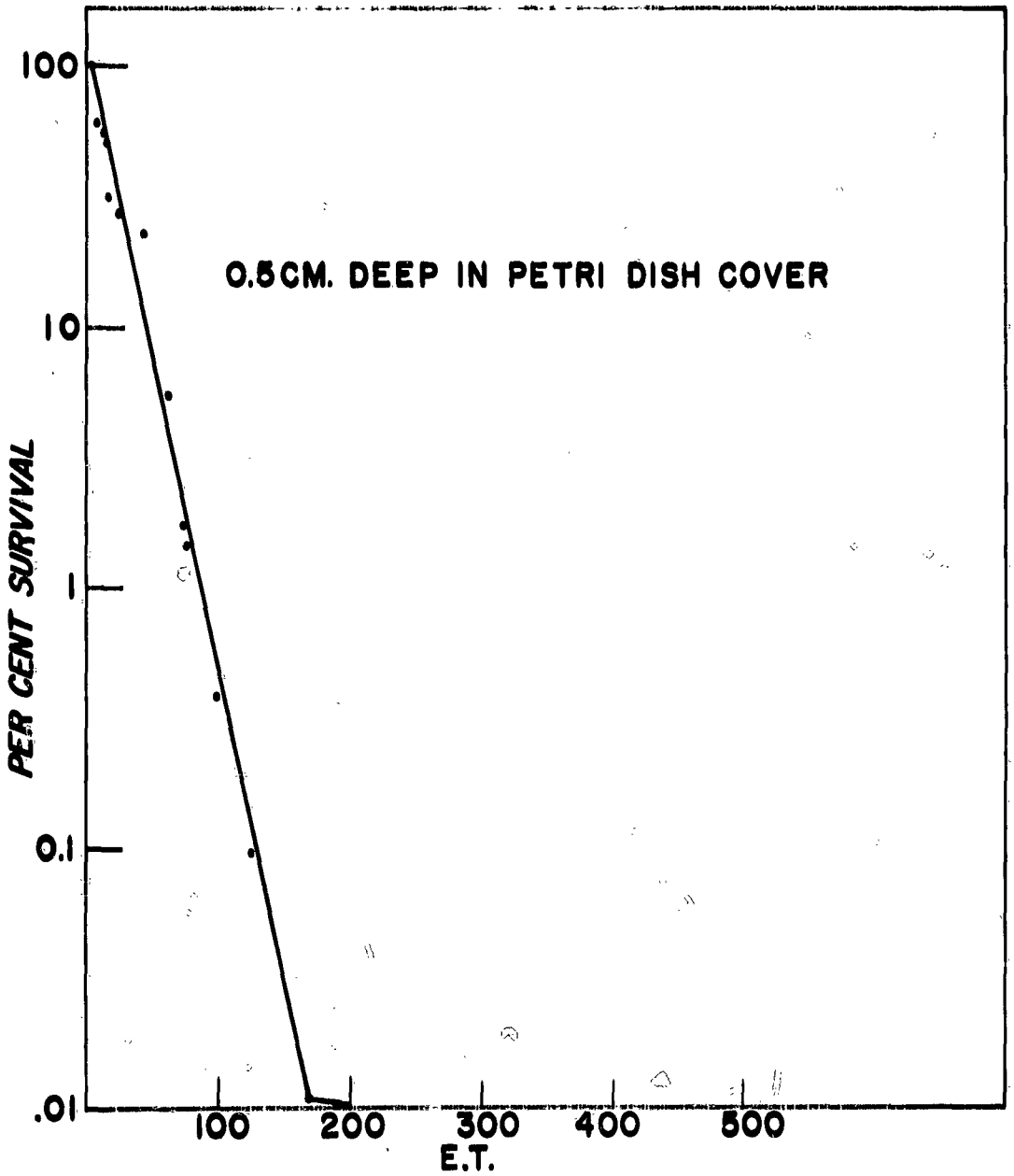

Figure 28. Survival of S. marcescens Cells in Distilled Water. Exposed to radiation from a 30-watt germicidal lamp.

Exposures were made of washed spores of Bacillus subtilis var. niger suspended in water. The results are shown in Table XXVI. An average of the counts on the control suspension in these experiments was 49 x 10^6 organisms per milliliter with the limits being 30 x 10^6 and 60 x 10^6.

TABLE XXVI. SUMMARY OF ET VALUES FOR 90, 99 AND 100 PER CENT INACTIVATION OF TEST ORGANISMS IN WATER AND ON SEVERAL DIFFERENT SURFACES

TEST ORGANISM	HOW EXPOSED	AVERAGE NUMBER OF CELLS EXPOSED	ET VALUES Per Cent Inactivation		
			90	99	100
E. coli	Agar surface	200	90.4	119	133
S. marcescens	Agar surface	200	54.4	11.5	164
S. marcescens	Glass surface	200	16.6	41.6	45.7
S. marcescens	Water (0.5 cm deep)	49 x 10^6	45	85	425
B. subtilis spores	Agar surface	200	292	478.8	638.4
B. subtilis spores	Glass surface	200	199.5	305.9	532
B. subtilis spores	Stainless steel surface	200	275	700	800
B. subtilis spores	Water (0.5 cm deep)	50 x 10^6	1600	2400	3200
B. cereus spores	Agar surface	240	333	550	750

b. On Surfaces

For determining inactivation of organisms on agar surfaces, some workers expose one half of an inoculated agar surface to radiations while the unirradiated half serves as a control. Tests were made using this method, but it was found to give inconsistent quantitative results when compared to the method of exposing the entire agar plate and using several inoculated, but unexposed, plates for controls. Therefore, the latter method was used in all experiments in which microorganisms were irradiated on agar surfaces.

Strains of Serratia marcescens, Staphylococcus albus, and Bacillus cereus were grown in broth. An aliquot of each culture was placed on glass, cardboard, copper, painted or unpainted pine wood and the surfaces exposed overnight to UV radiation. In each test, the panels were placed 90, 180, 210, and 266 centimeters from the UV lamps. The 266-centimeter figure was the distance from the ceiling lamp to the floor. Unexposed panels were used for controls and recovery was made by swabbing the surfaces with a damp cotton swab and streaking the swab on blood agar plates. It was found that after an exposure time of 18 hours, no viable cells of S. marcescens, S. albus, or B. cereus could be recovered from the exposed surfaces, while surfaces not exposed showed recovery of viable organisms.

In order to secure more quantitative data, the following experiments were undertaken: Cells of E. coli, S. marcescens, and spores of B. subtilis were washed twice in sterile distilled water and nebulized* onto glass, stainless steel, and various agar surfaces. The surfaces were inoculated by applying a constant number of squeezes to the rubber bulb of the nebulizer and using a standard distance between the nebulizer and surface. In this manner the surfaces were inoculated with approximately equal numbers of organisms. In each experiment two or three of the inoculated, nonirradiated plates were used as controls; the average counts being used as the final control. After exposure to radiant energy, nutrient agar was poured over the glass and stainless steel surfaces and the plates incubated with the control plates. Colony counts were made after 48 hours of incubation at 37°C.

The survival curves of these organisms, plotted against the ET values, are shown in Figure 29. Each point on the graph is an average of at least three tests which very closely duplicated themselves. The average number of colonies on the control plates was 200.

Table XXVI shows ET values for 90, 99, and 100 per cent inactivation of test organisms on surfaces and in water suspensions 0.5 centimeter deep.

c. On Air-Borne Organisms

Experiments were conducted in four rooms to determine the reduction in number of air-borne bacteria when two bare 30-watt hot cathode lamps, located in the ceiling, were turned on for one hour. The doors and windows of these rooms were closed and activity kept at a minimum during the tests.

The general procedure was to take two, 20-minute sieve air samples in the room before turning on the lamps, then three 20-minute samples while the lamps were on, followed by two more 20-minute samples after the lamps had been turned off. All samples were taken at the table

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* Vaponefrin Nebulizer, Vaponefrin Co., Upper Darby, Pa.

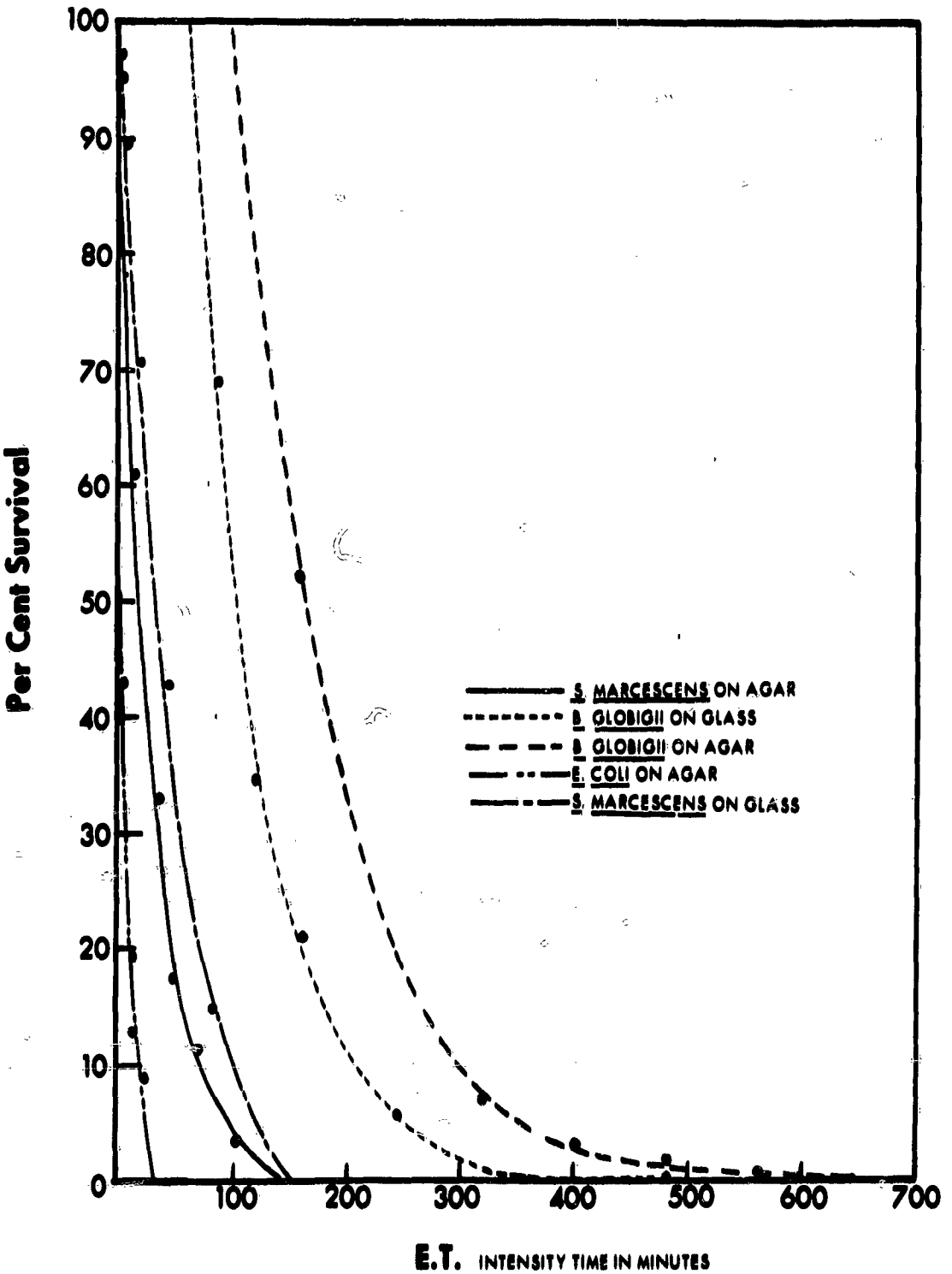


Figure 29. Survival of Organisms on Glass and Agar Surfaces Exposed to UV Radiation.

top level (180 cm from ceiling) and the samplers placed so as to be shielded from direct or reflected UV radiation. The results of a typical experiment conducted in an 1890 cubic-foot room are shown in Figure 30. In Table XXVII is shown the averaged results from experiments in four test rooms. The experiments in each room were repeated two or three times.

Table XXVIII is taken from Luckiesh (185) and shows the survival of irradiated "B. coli" in air. These data are rather typical of the average resistance of air-borne vegetative bacteria to UV radiation.

d. On Bacterial Spores

There is little doubt that bacterial spores are relatively resistant to UV radiation and that the order of magnitude of their resistivity, as compared with vegetative cells, is about the same as to heat and to some disinfectants. Koller (174) presented data showing the relative amounts of UV energy necessary to inactivate a vegetative organism and a spore forming organism:

	Microwatt-Seconds Per Sq Cm
B. coli on agar	6,600
B. subtilis on agar	60,000

The spore former, in this case, was about nine times as resistant as B. coli.

Table XXIX is taken from Table XXVI and shows the relative resistance of two bacterial spores as compared with two vegetative bacteria. From 3.3 to 5.4 times as much radiation was required to kill B. subtilis spores as for the two vegetative strains. From 3.7 to 6.1 times as much energy was required for B. cereus spores. These values are somewhat lower than those shown by Koller. Differences in UV source and type of measuring devices may be responsible for some of the discrepancy. This type of experiment has been repeated many times and it has been consistently true that bacterial spores are at least three to five times as resistant to UV radiation as vegetative bacteria.

C. EFFECTS ON VIRUSES AND BACTERIOPHAGE

1. Viruses

The application of most of the data of earlier workers who exposed viruses to UV radiation is of little value except to demonstrate that viral particles can be successfully inactivated. Monochromatic radiation was, for the most part, not used, and few intensity measurements were furnished. Ellis et al (75) reviewed some of these studies with viruses. Susceptible types are included in the list on page 109.

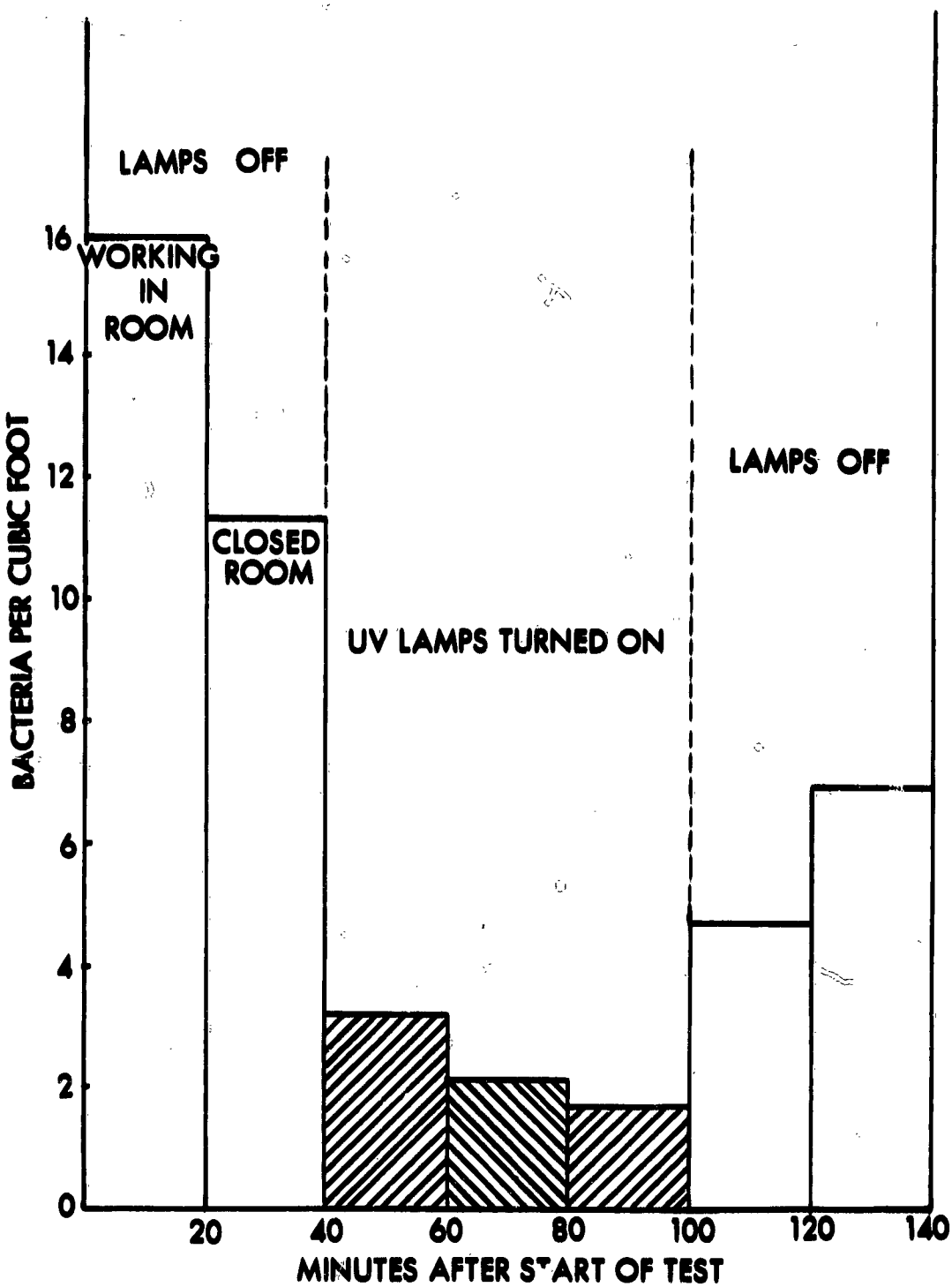


Figure 30. Concentrations of Air-Borne Bacteria in a 1890-Cubic Foot Room Before, During, and After Irradiation for One Hour with Two 30-Watt UV Lamps.

TABLE XXVII. REDUCTION OF AIR-BORNE BACTERIA BY UV RADIATION IN FOUR TEST ROOMS

ROOM NO.	CU FT IN ROOM	NUMBER OF 30-WATT LAMPS	AVERAGE BACTERIAL COUNT PER CU FT BEFORE LAMPS ON	LAMPS ON ONE HOUR						LAMPS OFF	
				1st 20 Minutes		2nd 20 Minutes		3rd 20 Minutes			
				Count Per Cu Ft	Per Cent Reduction	Count Per Cu Ft	Per Cent Reduction	Count Per Cu Ft	Per Cent Reduction	1st 20 Minutes Bacteria Per Cu Ft	2nd 20 Minutes Bacteria Per Cu Ft
1	1896	2	13.6	4.6	66.0	4.5	67.0	1.9	86.0	4.1	5.3
2	1890	2	17.7	10.4	40.0	7.4	58.0	4.4	75.0	7.4	11.0
3	375	2	33.0	4.4	87.0	2.5	92.5	2.7	91.8	4.2	6.6
4	1890	2	13.6	3.2	75.5	2.1	84.5	1.7	87.5	4.7	6.9
AVERAGES			19.47	5.65	71.0	4.1	79.0	2.7	86.0	5.1	7.45

TABLE XXVIII. UV INACTIVATION OF B. COLI IN AIR AT ORDINARY TEMPERATURES AND AT RELATIVE HUMIDITIES LESS THAN 40 PER CENT[a]

EXPOSURE, Microwatt-Minutes Per Sq Cm	PERCENTAGE KILLED
0.5	10
1.0	18
2.0	33
3.5	50
5.0	63.2
8.0	80
10.0	86.5
11.5	90
15.0	95
19.5	98
23.0	99
26.5	99.5
31.0	99.8
34.5	99.9
46.0	99.99

a. Luckiesh (185).

TABLE XXIX. RELATIVE AMOUNTS OF UV ENERGY REQUIRED TO INACTIVATE BACTERIAL SPORES

TEST ORGANISM	RATIO USING VALUE OF 1 FOR VEGETATIVE ORGANISM					
	E. coli			*S. marcescens*		
	Percentage Kill			Percentage Kill		
	90	99	100	90	99	100
B. subtilis spores	3.3	4.0	4.8	5.4	4.3	3.9
B. cereus spores	3.7	4.6	5.6	6.1	5.0	4.6

Tobacco mosaic virus has been shown by Hollaender (150) to be approximately 200 times more resistant to UV radiation than bacteria. Maximum action occurred at wave length 2600A. Aerosols of the influenza virus (Pr 8 strain) were found by Wells and Brown (307) to lose their infectivity for ferrets after exposure to radiations from a mercury arc.

Polson and Dent (239) used a high pressure quartz UV lamp to irradiate 11 strains of African horse-sickness virus. Although inactivation rates differed, all strains were susceptible to the lethal action of the radiations.

Carlson et al (42) found that treatment of water with artificial UV radiation was more effective in inactivating poliomyelitis virus than was exposure to direct sunlight or treatment with common water purification methods such as coagulation and sedimentation, sand filtration, absorption on inactivated charcoal, aeration, adjustment of pH and storage.

Data by Hollaender and Oliphant (153) showed that tobacco mosaic and chicken tumor 1 virus had maximum sensitivity at wave lengths shorter than 2300A while vaccinia virus, influenza virus, and bacteriophage showed maximum susceptibility at 2650A.

Studies with several strains of the rickettsiae of epidemic typhus, Rickettsia prowazeki, (2) indicated that irradiation with 2537A produces loss of respiratory activity and toxicity, in that order. Prolonged irradiation produced loss of both properties.

2. Bacteriophage

a. Literature Review

Bacteriophage active against Streptococcus lactis was found by Whitehead and Hunter (317) to be susceptible to UV radiation if sufficient exposure was given. Applemans (12) and Zoeller (328) determined that short exposure to UV radiation killed Shigella bacteriophage. The time required for the destruction by UV radiation of Shigella bacteriophage in solution was found to be influenced by the type of suspending liquid, the depth of the liquid, and the concentration of bacteriophage (214). According to Sutton (279) bacteria-free filtrates containing bacteriophage active against Streptococcus cremoris were destroyed by radiation in six minutes at a distance of three inches from an UV lamp.

Gates (94) showed a culture of Staphylococcus aureus to be more susceptible to the lethal action of UV radiation than its homologous bacteriophage. E. coli bacteriophage, on the other hand, was noted by Latarjet and Wahl (178) to be two to six times more sensitive to UV irradiation than the homologous strain of E. coli. However, the bacteriophage was more resistant when a mixture of bacteriophage and cells was irradiated.

Anderson (9) found that bacterial cells unable to form colonies after UV irradiation were also unable to support the growth of the bacterial virus. Apparently irradiation of host cells caused the liberation of a virus-inhibiting substance, reduced the burst size of the host and inactivated the virus and its host. Cells of E. coli apparently lose their ability to liberate bacteriophage after irradiation, because of the inactivation of the intracellular virus (193).

Greene and Babel (107) studied the suitability of UV radiation to control Streptococcus lactis bacteriophage in dairy plants and determined the exposure times and distances from the source that would be necessary to obtain complete destruction. Although intensity measurements were taken at the surface of the UV lamps, these authors neglected to record the intensity present at the exposure surface. The lamps used were very weak sources of UV radiation.

b. Experimental Studies on Agar Surfaces

Bacterial-free suspensions of bacteriophage T-3 for E. coli B were spread on the surface of tryptose phosphate agar plates, and the plates were exposed to the radiations from a 15-watt, hot cathode UV lamp. The exposures were made at a distance of 12 inches from the UV source, and the length of the exposures was accurately determined with the aid of a stop watch. After the exposure, three milliliters of melted agar containing a suitable number of host bacteria, were layered over each exposed surface. The plates were incubated for 18 hours at room temperature and the number of typical phage plaques counted. The total number of phage particles exposed in each test was determined by plaque counts on the original phage suspension.

Each test was repeated four times. For each exposure, the average number of plaques counted was determined and expressed as a percentage of the number of phage particles originally exposed. The radiation dose received in each exposure was calculated in microwatt-minutes per square centimeter (ET value). In the four tests, the average number of phage particles exposed per plate was 2,500.

Table XXX shows the results obtained. These data were used in the preparation of the survival curve shown in Figure 31. By comparison with the survival curves for vegetative bacteria shown in Figure 29, coliphage appear slightly more resistant than its host.

c. Experimental Studies on Air-Borne Clouds

Bacteriophage T-3 was used as a simulant for pathogenic viral strains because it is a submicroscopic particle in the same general size range as many human viruses, and it attacks a specific host. These were made to determine the effectiveness of 2537A UV radiation against airborne particles of coliphage. Air locks, door barriers, and other installations equipped with germicidal lamps are intended to act mainly on aerial

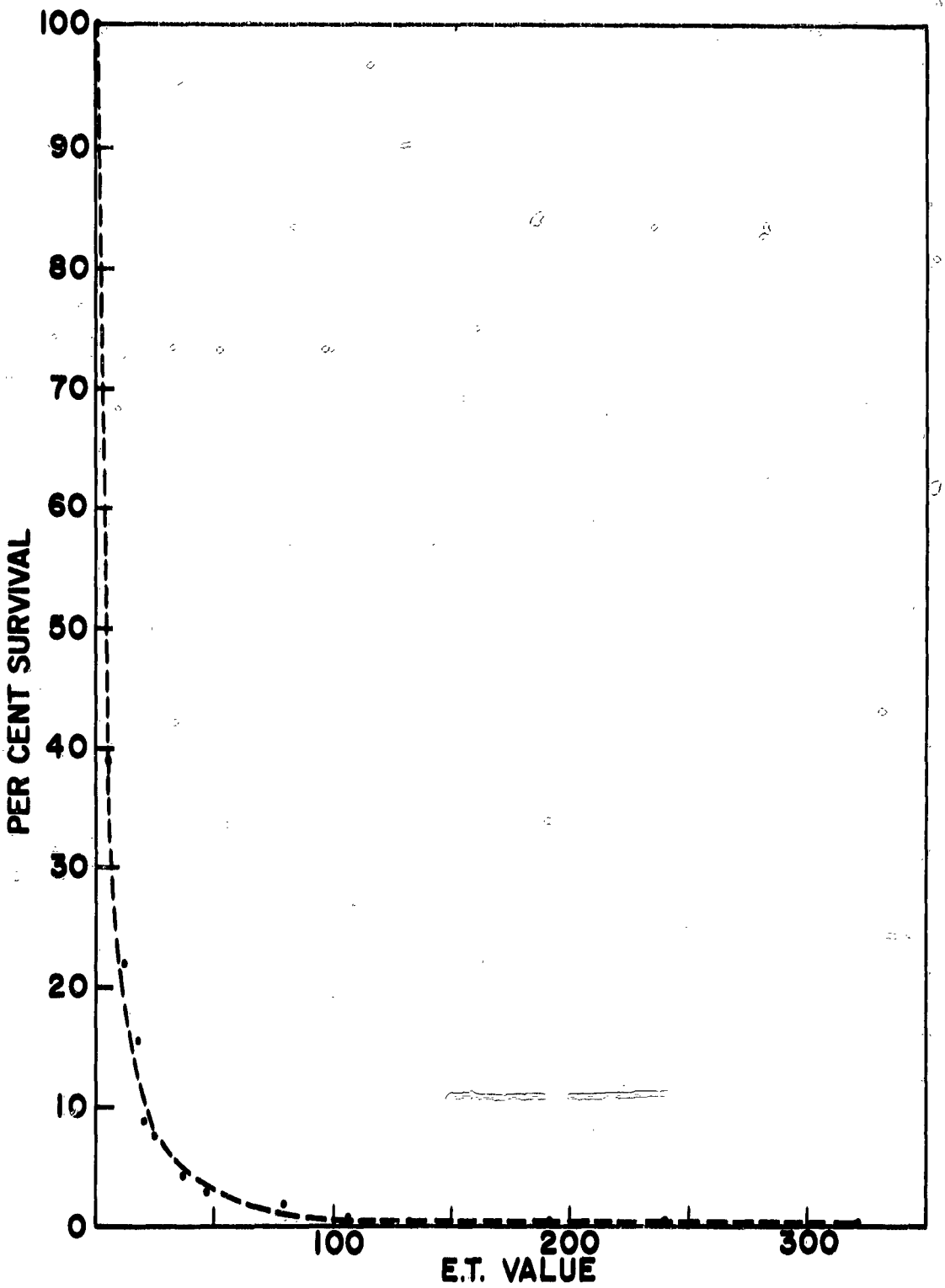


Figure 31. Survival of T-3 Coliphage on Agar Surfaces Exposed to UV Radiation.

microorganisms in separating contaminated areas from clean areas. Excellent results have been obtained with air-borne bacteria, but less evidence has been presented for the virus.

TABLE XXX. INACTIVATION OF T-3 COLIPHAGE ON AGAR SURFACES WITH UV RADIATION

EXPOSURE TIME, Seconds	ET VALUE, Microwatt-Min Per Sq Cm	PER CENT KILL
2	5.32	61.00
4	10.64	78.35
6	15.96	84.46
8	21.28	91.78
10	26.60	92.25
14	37.24	95.62
18	47.88	96.96
20	53.20	97.07
30	80.00	98.71
40	106.60	99.46
50	133.20	99.86
60	160.00	99.90
120	320.00	99.99

The tests were conducted in a closed metal chamber having a volume of 5.7 cubic feet and equipped with one 15-watt, cold cathode lamp. Exhaust air from the chamber was sterilized by passage through a filter. The cabinet was equipped with two outlet sampling lines and one inlet air line.

Filtrates of coliphage containing 1.6×10^8 phage particles per milliliter were prepared in a protolysate broth solution. The stock suspension was sprayed from a Vaponefrin atomizer to produce the phage clouds. Air samples were taken with critical orifice impingers. Air samplers were connected to the sampling lines of the aerosol chamber and operated at the rate of 14 liters per minute. The general test procedure was as follows:

(a) Approximately 1.5 milliliters of the phage suspension were atomized into the test chamber.

(b) The UV lamp was turned on for two minutes.

(c) Two 5-minute air samples were taken simultaneously while the UV lamps continued to burn.

Control tests were exactly the same except that no UV radiation was used. The number of phage particles collected was determined by plaque counts in tryptose phosphate agar using the susceptible E. coli B host strain. In one test, samples of the cloud were taken with a sieve-type air sampler.

UV intensity readings were made in the chamber. Intensities of approximately 400 microwatts per square centimeter were obtained in the top of the chamber, and at the bottom, near the sampling outlets, the intensity was 180 microwatts per square centimeter.

In Table XXXI are recorded the results of six tests showing the number of phage particles collected during off and on periods and the per cent reduction by the UV radiation. Complete inactivation occurred when clouds of T-3 coliphage, in a concentration of about 10,000 phage particles per cubic foot, were exposed to the UV radiation for a two-minute exposure period followed by a five-minute sampling period (total of seven minutes). Higher aerosol concentrations, up to 8.68×10^6 particles per cubic foot, were reduced over 99.9 per cent by similar exposures.

These tests demonstrate the susceptibility of T-3 coliphage in the air-borne state to the germicidal action of UV radiation in the 2537A range.

TABLE XXXI. INACTIVATION OF AIR-BORNE T-3 COLIPHAGE WITH UV RADIATION

TEST NUMBER	NUMBER OF PHAGE PARTICLES ATOMIZED	NUMBER OF PHAGE PARTICLES COLLECTED PER CU FT OF AIR SAMPLED		PER CENT REDUCTION BY UV RADIATIONS
		Ultraviolet Off	Ultraviolet On	
1	3.0×10^8	2.64×10^6	2.44×10^3	99.908
2	3.0×10^8	8.68×10^6	1.44×10^3	99.984
3	3.0×10^8	4.96×10^6	1.92×10^3	99.961
4	3.0×10^7	1.39×10^4	0	100
5	3.0×10^6	6.56×10^3	0	100
6	3.0×10^5	125*	0*	100

* Sieve sampler used.

D. EFFECTS ON FUNGI

Koller (174) states that molds and yeasts are 100 to 1000 times as resistant to the lethal action of UV radiation as are bacteria. Nagy (218) has shown that variation in resistance correlates approximately with the degree of pigmentation of the molds spores. Some of the colorless molds and yeasts are only slightly more resistant to UV radiation than most bacteria, Table XXIV. Colored mold spores are many times more resistant. It was also shown that, at certain stages in their life cycle, molds, just as bacteria, are more susceptible to UV radiation. Exposing the mold spore to a low intensity of radiation for many hours required from one-fifth to one-tenth the energy to destroy the organisms as similar organisms exposed in a period of minutes to the same total amount of energy.

The treatment of tobacco leaves with UV to inactivate molds has been tried occasionally. Dorcas (66) pointed out that the color of the leaves is altered by UV treatment and that other changes occur in the quality of the tobacco.

Using a mercury tungsten arc, Fulton and Coblents (91) exposed spores of 27 species of fungi to UV radiation for one minute. Spores of 16 of the species were completely inactivated and four species showed a survival of less than one per cent. UV radiation in the Schumann region (less than 2000A) was found by Johnson (163) to be destructive to the upper layers of the mycelium of Collybea dryophila, Sclerotium batacicola, and Fusarium batacia. Ramsey and Bailey (243) found that irradiation by a quartz mercury arc stimulated the formation of spores in cultures of Macrosporium tomato and Fusarium cepae. The spores of Puccinia graminis tritici were more easily inactivated in a water suspension than in a dry state when irradiated with UV radiation by Dillon-Weston (65). Fatty or waxy secretions undoubtedly offer protection to some species of fungi.

Luckiesh et al (192) reported the UV dosages required to destroy 95 per cent of various air-borne mold spores.

	Microwatts Per Sq Cm to Inactivate 95 Per Cent
MOLD SPORES	
Mucor mucedo	1250
Penicillium chrysogenum	1150
Scopulariopois brevicaulis	1450
Cladosporium herbarum	1350
Aspergillus amstelodami	1450
YEAST	
Torula sphaerica	50

Studies also showed that when two bare 30-watt hot cathode UV lamps were burned in a room of approximately 3000 cubic feet, for two hours, the number of air-borne Penicillium chrysogenum spores was reduced by 85 to 87 per cent.

E. TOXIN-DESTROYING POWERS

Most available information concerning the action of UV radiation on bacterial toxins gives little information as to the intensity of the radiations or the wave length used. These studies have been conducted, for the most part, in special apparatus designed either for the preparation of antigens (110) or for irradiating the blood of individuals with bacterial infections (100). Little success was experienced in attempting to inactivate toxins suspended in blood. The radiation source was a high-pressure, water cooled, mercury quartz lamp.

The following data, from Habel and Sockrider (110) indicate the relative sensitivity of Shigella dysentery toxin to UV energy, as compared with a bacterial suspension and a virus emulsion.

Antigen	Exposure Time to UV Necessary to Inactivate Antigen	Ratio
Typhoid bacterial suspension	10 seconds	1
Rabies virus emulsion	10-80 seconds	1-8
Shigella dysentery toxin	1200 seconds (20 min)	120

The bacterial toxin was 120 times as resistant to the destructive action of UV radiation as was a bacterial suspension.

Blundell et al (32) exposed four bacterial toxins in blood and in saline to UV radiation in a Knott hemo-irradiator. "Each 10 milliliters of the fluids received a 10-second exposure of UV irradiation which varied in wave length from 2399 to 3654 angstrom units." Under the test conditions, UV radiation had no detoxifying action on the four toxins when they were suspended in blood. In saline solution, a slight detoxifying action was noted on tetanal and diphtherial toxins, but not on staphylococcal toxin or scarlet fever streptococcal toxin.

In general, it can be stated that bacterial toxins are difficult to inactivate with UV radiation; inactivation occurs only after long exposures to high intensities.

X. PRACTICAL USES OF ULTRAVIOLET RADIATION

A. LITERATURE REVIEW

Since the advent of the low pressure mercury vapor lamp there has been renewed interest in the application of UV radiation for health protection and product protection. Special devices have been made utilising the bactericidal, fungicidal, virucidal, toxin-inactivating, therapeutic (39), and ozone producing properties of this radiation. Some of these devices are of considerable value in preventing infections or preserving foods and other products. Others are of questionable value because of insufficient radiation or lack of understanding of the properties of germicidal energy. A brief survey of some of the applications of germicidal radiation will be presented.

1. Toxins, Viruses, Vaccines, and Blood Plasma

The early work on molds, toxins, and filtrates was summarized by Welch (303) in 1930. Unfortunately, the wave lengths and the quantity of radiation were not always reported.

In 1932, Mayer and Dworski (203) reported the action of UV radiation on Mycobacterium tuberculosis. A suspension of tubercle bacilli containing 2.7×10^6 organisms per milliliter was killed within four minutes by absorption of 1.42×10^9 ergs per second per square centimeter from the unfiltered radiation of a quartz mercury arc. Exposure to 5.93×10^6 ergs per second per square centimeter for 25 minutes did not alter the acid-fast staining qualities of the organisms. Trotskii et al (280) in 1935 found that bacteria exposed to UV radiation lost considerable virulence for laboratory test animals but retained their antigenic and immunising characteristics. The use of radiation as a means of inactivating bacterial and viral suspensions in the preparation of immunising antigens came into prominence with the work of Hodes et al (148) on rabies virus. Irradiation of the virus for 45 minutes produced suspensions which were avirulent for mice and which possessed ten times the immunising properties of chloroform treated virus and 500 times the activity of phenolized vaccines.

These and other reports led to the development by Oppenheimer and Levinson (231) in 1943 of an apparatus for exposing bacterial and viral suspensions to UV radiation. Levinson et al (184) in 1945 described the production of rabies and St. Louis encephalitis virus vaccines by this method. Milzer et al (213) of the same laboratory simultaneously announced the production of a radiation inactivated poliomyelitis vaccine. Earlier, Taylor et al (282) in 1941 showed that the absorption curve for equine encephalomyelitis virus resembles the absorption of radiation by bacteria. This virus was inactivated by the same order of magnitude of radiation as is required to inactivate Serratia marcescens. Sarber et al (263) used UV radiation to prepare experimental vaccines for tuberculosis.

Hollaender and Oliphant (153) in 1944 showed that the action spectrum for influenza virus and E. coli were the same. About 20 per cent more energy was necessary to inactivate influenza virus than to destroy E. coli.

In 1946 Oliphant and Hollaender (229) showed that hepatitis virus in human blood plasma or serum can be destroyed by UV radiation. Wolf et al (322) in 1947 and Blanchard et al (28) in 1948 also described the inactivation of this virus in human plasma. In 1950 Cutler et al (53) irradiated human blood plasma to destroy the virus causing homologous serum jaundice.

Variations of the preceding apparatus using ultraviolet lamps have been published. Habel and Sockrider (110) described an apparatus which incorporated a 15-watt, hot cathode, germicidal lamp. The apparatus of Habel and Sockrider was modified by Bozeman et al (36) for use in serum and vaccine production. Benesi (26) in 1956 described a high speed centrifugal filmer for the irradiation of liquids. The liquid blood plasma or bacterial or virus suspensions are irradiated in this type of apparatus in a very thin film of approximately 0.25 millimeter. The absorption of UV by blood is much greater than the absorption by water as shown by the coefficient of absorption of 2537A radiation for blood which is approximately 71 per centimeter compared with water which is generally less than 0.2 per centimeter.

Experience with the use of UV for the treatment of blood plasma to inactivate the causative agent of serum hepatitis demonstrates the importance of correct dose in practical applications of UV. Methods for irradiating thin films of plasma with 2537A radiation established prior to 1950 (28,322) appeared favorable because a dose not affecting the serum proteins appeared effective in reducing the hazard of hepatitis. Experience between 1950 and 1953 showed that administration of irradiated blood or blood products frequently resulted in serum hepatitis (20,108,162,216), although it was later reported (217) that such cases exhibited longer incubation periods and milder illnesses than cases developing after the injection of nonirradiated plasma. Murray et al (217) found that the dose necessary to produce sterilization of the plasma caused extensive changes in the plasma protein. Subsequently other treatment methods have been employed in which UV irradiation is combined with other means of disinfection and in which other viruses have been used as a test agent in determining sterility.

Smolens and Stokes (271) added T4R coliphage to normal human serum which was then irradiated in a Dill apparatus (J. J. Dill Company, Kalamazoo, Michigan) and also was treated with beta-propiolactone at several different concentrations. UV treatment alone reduced the phage count from 87 x 10^7 to 1070 particles per milliliter of serum. Addition of 1.5 milligrams of beta-propiolactone per liter of serum inactivated the remaining phage in 16 hours. Hartman et al (132) also used UV irradiation in conjunction with beta-propiolactone to treat plasma samples to which had been added 10 per cent suspensions of eastern equine encephalitis or encephalomyocarditis virus. Most of the viral particles were inactivated by the treatment. Amounts of beta-propiolactone sufficient to give consistant virus inactivation damaged the plasma proteins.

Buttolph* (151) has suggested the use of Sarcina lutea as a test organism for hepatitis virus because the characteristic packets of cells may simulate clumps in plasma wherein viral particles may reside. Storage of irradiated plasma for prolonged periods at room temperature (3,4) is one method presently used to render plasma safe for medical use.

In 1934, Knott and Hancock (172) published a method for irradiating the blood of individuals suffering from bacterial infections. The irradiating machine (commercially known as the Knott-Hemo-Irradiator) was designed so that blood from the patient was circulated in a closed, tubular system through a quartz irradiation chamber. The radiation source was a water cooled mercury quartz lamp. The wave length of radiation emitted from the lamp varied from 2399A to 3654A. Barger and Knott (18) summarized a list of diseases which can be controlled by this method of irradiation. Blundell et al (32) in 1943 demonstrated that bacteria and toxins in the blood are not effectively destroyed by this method.

2. Water Sterilization

Ultraviolet energy from high pressure mercury arc lamps has been used since 1909 for the sterilization of water (75). A number of cities in Europe as well as in this country have had installations capable of handling as much as three million gallons of water per day. As late as 1935, UV lamps were installed in England and Germany for the sterilization of water in swimming pools and city water supplies. Since the cost of this method of treatment greatly exceeded that of chlorination or ozone treatment, its use became limited to special applications. Introduction of the low pressure mercury discharge lamps has revived this application.

Preliminary studies on the disinfection of water with low pressure discharge lamps were published by Luckiesh and Holladay (186) and Luckiesh et al (190). A small practical unit was described by Ricks et al (248).

Factors of importance in treating waters with UV radiation are the coefficient of absorption of 2537A and the temperature of the water. Temperature is important because it may limit the UV output of mercury vapor lamps. Thus at 50°F the UV output of a high intensity lamp will be only 20 per cent of that obtained at the optimum of 105°F.

There have been various statements regarding the susceptibility of organisms in water as compared to air-borne or surface-borne organisms. Luckiesh (185) on the basis of his studies indicated that approximately five times as much energy was required to inactivate E. coli in water as for the same organism on agar surfaces. Others have stated that 40 to 50 times as much exposure is necessary to disinfect water as compared to organisms suspended in dry air (98). Accurate comparison of the various studies is difficult because of the factors of temperature and absorption coefficients. Consequently, these factors must always be determined and their influence noted. Another obvious consideration is that baffles and

* Cited in Hollaender.

other devices must be included to insure that all water will receive sufficient radiant energy. Table XXXII shows results furnished by Nagy of experiments conducted in a six-inch diameter cylinder containing lamps emitting a total of 9.62 watts of 2537A. The water at 52°F contained 1200 E. coli organisms per milliliter before treatment.

Several models of an UV water "sterilizer" in which high-intensity lamps are used are commercially available.* Units capable of treating up to 166 gallons per minute are advertised. Tests conducted with E. coli showed that the units effectively reduce the bacterial count when water with a low coefficient of absorption is used. When properly used with water having an E. coli count of approximately 1200 organisms per milliliter, the units will give at least 99.99 per cent kill of the organisms.

TABLE XXXII. INACTIVATION OF E. COLI IN WATER IN A SIX-INCH DIAMETER CYLINDER

GALLONS PER HOUR	PER CENT INACTIVATION OF E. COLI
1480	100
2239	99.6
2768	99.5
3480	99.2
4500	98.5
5382	94.0

Conditions: Water temperature - 52°F
Coefficient of absorption of water - 0.19 per cm
Test organisms - 1200 E. coli organisms per ml of water
UV lamp output - 9.62 watts of 2537A

Cortelyou et al (49) studied the effectiveness of a small water purifier on several water-borne bacteria. The purifier unit** utilizes a 4-watt hot cathode lamp mounted in a metal head which is designed to attach to a screw-on glass jar. Exposure of water-borne particles at four flow rates between 0.5 and 2 quarts per minute is obtained by means of baffles. Dosage measurements with a depreciated lamp indicated that the expected values for water-borne bacteria at the four flow rates was at least 300 to 1240 milliwatt-minutes per square foot. The highest dosage showed 100 per cent destruction of as many as 20,000 Salmonella typhosa organisms per milliliter of treated water. E. coli in a concentration of 13 x 10^4 organisms

* Aquafine Corp., 1005 S. Santa Fe Ave., Los Angeles 21, California.
** URF UV water purifier, Model IC 196, URF Products, Inc., River Forest, Illinois.

per milliliter were 99.97 to 99.99 per cent removed at the four flow rates. From 99.69 to 99.99 per cent of Staphylococcus aureus in an initial concentration of 34 x 10^3 organisms per milliliter were likewise inactivated. Lower efficiencies were obtained with Bacillus subtilis suspensions, mixed species of organisms, and naturally polluted waters. The dose levels given agree substantially with other data presented in this report.

A number of larger UV water treatment apparatus using one to four or more G36T6 UV lamps are also available (1,2,207). The newer units made by these companies use quartz tubes to enclose the lamps so that the temperature of the water does not influence the output of the lamp. From 300 to 3,000 gallons per hour of potable water can be obtained. They have been used in the dairy, brewing, and pharmaceutical industries where chlorine could cause oxidation of the product. The efficiency of any UV water sterilizer is dependent upon the amount of radiation, the degree of mixing, the turbidity of the water, and the transmission of UV through the water. It is apparent, however, that UV purifier units for water cannot be depended upon to completely inactivate all forms of microscopic life, especially if some turbidity is present.

Calculations have been made for the rates of disinfection of waters of various coefficients of absorption when seeded with vegetative bacteria. In general it was found that potable water usually can be produced by the application of one watt of 2537A radiation for each 100 gallons per hour of water flow, providing the water has had an absorption coefficient of 0.10 per inch or less.

3. Hospitals

Since surgery was first performed, air-borne organisms have been a serious problem. Many post-operative infections and deaths result from these air-borne organisms. Lister sprayed carbolic acid to eliminate infectious organisms from the air but this is not possible today.

Although bactericidal lamps are used in few hospitals, a considerable amount of information is available describing hospital applications of UV radiation. In addition, many of the applications found worthy of use in infectious disease laboratories obviously can be used to advantage in the hospital. As with other applications, proper and successful application of UV radiation in hospitals demands (a) use of the proper intensities and exposure times, (b) proper maintenance and replacement of UV lamps, and (c) personnel protection, where necessary.

As mentioned elsewhere, it is also necessary to have an understanding of the limitations of the use of UV radiation. UV radiation is not the "solver of all problems," and in no instance should its use be substituted for proper aseptic technique and good housekeeping.

In presenting a brief discussion of the hospital applications of germicidal radiation, it is pointless to do more than mention the numerous opportunities which may exist for the spread of infectious diseases in such institutions. Infections have been spread by the air, through clothing, and by thermometers, instruments, and other fomaties. Hospital-incurred infections can occur in the operating room, infant wards, patient wards, hospital laboratory, laundry or any other place in the hospital. Recently, epidemics of staphylococcal infections have been noted in many hospitals.

Based on a nine year study of operative wounds, Meleney (209) estimated that between 30,000 and 60,000 viable organisms fell upon the "sterile" field during the course of an hour's operation. These figures, however, are extremely high for present day hospitals. In a survey of 37 operating rooms in 17 states, Hart (122) demonstrated the universal presence of pathogenic bacteria in operating rooms during occupancy. Most of the infections of clean wounds were believed to be caused by air-borne staphylococci falling into the wound or on sterile supplies. The number of organisms settling on a Petri dish during an operation varied from 21 to 188 with an average of 67. An experiment carried out using Petri plates of blood agar plated with hemolytic Staphylococcus aureus were placed at an operative site and exposed from one to three minutes to UV radiation. Air-borne bacteria were reduced from 95 to 100 per cent. The intensity of radiation did not cause an appreciable burn during an exposure of ninety minutes. Hart (120) first discussed the feasibility of an operating room with air currents from other parts of the hospital eliminated and using direct sunlight. Artificial sources of UV radiation were used to prove or disprove that bacteria in the air were the chief source of danger and "unexplained infections" and could be controlled by the elimination of air-borne organisms.

Hart and Jones (128) reported that pathogenic bacteria exhaled by the operating room personnel were the predominant cause of infections in "clean incisions." Hart (122) reduced the number of bacteria by rigid isolation in the operating rooms. Because of the low filtration efficiency of surgical gauze masks (109), large heavy masks were worn over the nose and mouth by all occupants of the operating rooms at all times, regardless of whether an operation was in progress. By irradiating the air with UV radiation, it was possible to obtain less than one colony per open Petri plate per hour of exposure in the operative field. Unexplained infections in "clean incisions" are practically nonexistent.

In a series of publications starting in 1936 and extending over a period of years during which time thousands of operations were done, Dr. Hart and his associates (121-129,323) have clearly demonstrated the benefits of using UV radiation in the operating room. The reduction of pathogenic organisms in the operating room will have the following effects:

(a) Reduction of post-operative infections by 85 per cent.

(b) Eliminates occasional death from infection.

(c) Reduces the post-operative temperature.

(d) Reduces the duration of post-operative temperature.

(e) Improves wound healing.

(f) Lessens systemic reactions.

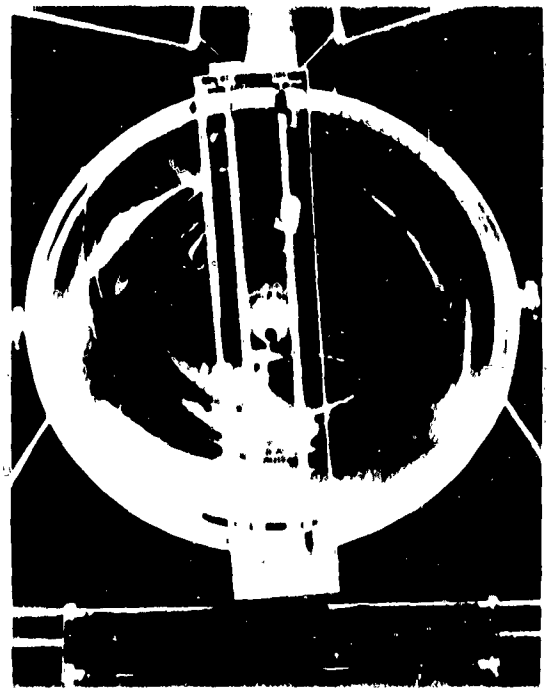
(g) Shortens convalescence.

These effects were most pronounced in patients subject to extended surgery.

Rentschler et al (247) and Sharp (266-268) have shown that less than 10,000 microwatt-seconds of 2537A radiation (166 microwatt-minutes) will destroy most common pathogenic organisms. What effect would this energy have on living tissue? Hart et al (130) have shown that intensities of about 20 microwatts per square centimeter cause no demonstrable harm to skin, peritoneum, meninges, or other tissues exposed during an operation. Kraissi et al (176) and Fraser (89) demonstrated that microorganisms are destroyed before injury to tissue occurs. Odom et al (227) exposed the brains of twelve dogs to an intensity of 18 microwatts per square centimeter for as long as 30 minutes with no pronounced effect on the tissue.

Extensive use of bactericidal radiation has been made in construction of the new Duke University Hospital in Durham, North Carolina. All of the air and personnel in the operating theaters are exposed to direct radiation (Figure 32). A preparation room designed to supply all of the operating rooms is also irradiated with direct bactericidal radiation (Figure 33). Protection of the face and neck is necessary when direct radiation is employed (Figure 34). A one-half hour exposure of bare skin to an intensity of 20 microwatts per square centimeter will produce a mild erythema. Simple protective clothing and eye shields are used by the operating team. This has not been found to be a problem at this hospital.

Another method of irradiating air in an operating room is to use indirect radiation. UV lamps in specially designed fixtures are hung on the wall in a manner so that only the upper air of the room is irradiated. The recommended average intensity of 2537A radiation in the upper air zone is 50 microwatts per square centimeter. The fixtures are hung 6½ to 7 feet from the floor. The normal circulation of air in the room exposes most air-borne organisms to the high intensity radiation in the upper portion of the room. The average reduction of air-borne organisms is about 60 to 70 per cent. Published figures are not available on the reduction of the incidence of infection of clean wounds by this method. Based on previous data it would be logical to assume that the reduction of infection would be proportional to the reduction of air-borne organisms.



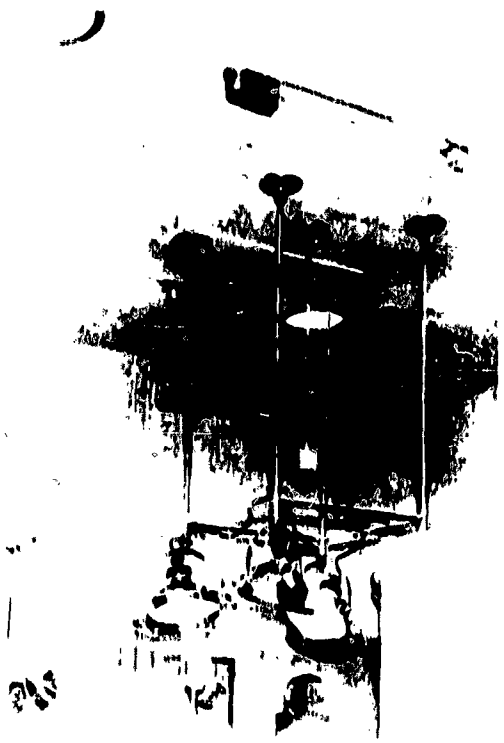


Figure 32. Duke University Operating Room. (FD Neg C-5824)

Figure 33. Duke University Preparation Room.
(FD Neg C-5825)

Figure 41 [illegible] Clothing Worn at Duke University. (FD Neg C-5826)

Special fixtures and cabinets with UV lamps have been used by Hart (119) and by Post (240,241) in eye surgery, to protect sterile instruments from air-borne organisms.

Generally, more attempts are made to supply sterile air to operating rooms than other sections of a hospital. Robertson and Doyle (254) have shown that cubicles in an infant ward had an average of 61 organisms per cubic foot. A large ward had an average of 360 organisms per cubic foot, while tests in an outpatient department had an average of 407 organisms per cubic foot but some days the counts were as high as 706 organisms per cubic foot. A series of tests by Robertson et al (256) showed that barriers of UV radiation were effective in preventing the spread of artifically introduced bacteria from cubicle to cubicle. Robertson et al (255), in a two and one-half year study on the use of curtains of UV radiation and upper air irradiation in a children's hospital, also showed that there was a reduction in the number of respiratory infections in infants. Higgons and Hyde (143,144) in a three-year study, demonstrated that UV radiation in a children's hospital reduced the incidence of respiratory infection by as much as 33 per cent. Friedoriszick (90) showed that indirect UV radiation in a children's hospital reduced to a considerable degree the infections of upper respiratory tract and their complications.

UV lamps have been employed in tuberculosis hospitals. MacVandiviere et al (198) demonstrated that indirect ultraviolet radiation in a hospital room destroyed over 70 per cent of air-borne organisms, some of which were M. tuberculosis.

Recently Riley et al (251,252) have conducted experiments in a tuberculosis ward to evaluate the value of UV radiation in preventing the air-borne spread of M. tuberculosis. A curtain of radiation is used to prevent the spread of air-borne tubercle bacilli from the patient's rooms to other portions of the hospital, and upper air irradiation is used in each patient's room. As a testing procedure all of the air exhausted from the rooms is passed to a chamber in the attic where guinea pigs are housed. Preliminary tests with sprayed tubercle bacilli demonstrated the effectiveness of UV radiation. Control tests were conducted in the ward with the UV turned off. The authors hoped to relate a quantum of infection for a nurse to the rate of guinea pig infections. From the results of the first several months of the control test it was estimated that one guinea pig infectious dose was contained in each 12,000 cubic feet of ward air. Further experiments are in progress to determine the effect of UV radiation on the infectiousness of the ward air.

High intensity radiation has also been used in some hospital autopsy rooms to destroy organisms on the tables, floor and in the air.

Rosenstern (261), in an adoption nursery (infants one to three months) of 36 cribs, compared three systems for the prevention of respiratory contagion in the cradle. They were (a) air conditioning, (b) air conditioning plus UV barriers in front of cubicles, and (c) barrier units having each cubicle closed off and with a separate air-conditioning system.

Clinical observations over a period of two years showed that air-conditioning methods did not prevent the spread of respiratory infection whereas the other two methods efficiently prevented cross-infection.

McKhann et al (206) reported that chicken pox failed, except in one case, to spread from 12 cases treated over a period of five months, in an isolation ward to 120 other children on the same floor who were separated from the infected room by an UV barrier.

Green et al (106) were engaged in a study of the effectiveness of UV radiation in a home for infants and children when an epidemic of chicken pox occurred. At that time they had under study two infants' wards, one with and the other without UV lamps. Ninety-seven per cent (165 of 170) of the children who were housed in the unirradiated main building and 18 of 19 in the unirradiated control ward contracted chicken pox. In the irradiated ward not a single case of chicken pox developed. It is interesting that a night nurse in the latter ward also cared for children in the adjoining ward where the incidence of infection was nearly 100 per cent.

4. Schools

Wells (305) reported that for three successive years, young children in UV irradiated rooms of a school were spared not only from chicken pox but from mumps as well, when these diseases were prevalent in other parts of the school. He also has shown (304) that the use of UV radiation in school rooms housing primary classes gave a very marked reduction in the number of cases of measles as compared with a similar group housed in unirradiated rooms.

The school tests of Wells were duplicated in a modified form by many investigators. Perkins et al (235) reported that UV radiation modified the spread of measles. Bahlke et al (16) observed differences in the rate of spread of chicken pox but could not find that the radiation had any effect on the spread of mumps. Wells and Holla (309) reported that UV lamps were effective in disinfecting the air in the school room during the winter months but ineffective during the moist spring months. It should be noted that the quantity of bactericidal radiation in these tests was less than that used in other experiments (305). The amounts of radiation used by other authors was also different so that it is difficult to compare the results of the various investigators. Gilcreas et al (103) reported that they observed, over a period of four years, an average of 42 per cent reduction of the total number of air-borne bacteria in school rooms with UV radiation. Downes (67), however, could not find evidence that UV radiation in schools effected the incidence of illness. However, in these studies only two 30-watt germicidal lamps in reflector fixtures were used per school room. Luckiesh et al (191) showed that four such lamps should be used to obtain a reasonable germicidal effect in a school room. Gelpherin et al (96) also observed only a minor reduction of respiratory diseases of children in schools irradiated with UV radiation. Gilcreas et al (104) later reported

a reduction of microorganisms in the school room without a reduction of the incidence of disease. They believed that the major transmission of disease occurred on school buses and outside of school hours. Air in the buses contained from 620 to 3780 organisms per cubic foot with an average of 980. The average number of organisms in the school room was found to be 34 per cubic foot of air.

The Medical Research Council, England, (207) reported the results of a three-year study of UV lamps in schools. The "examination of individual causes of absenteeism suggested that irradiation probably reduced the number of absences due to certain diseases by amounts between 15 to 45 per cent." The numbers of air-borne hemolytic streptococci counted over a period of six months were reduced by about 80 per cent while the over-all reduction in air-borne organisms was about 70 per cent. The radiation did not appear to reduce the large group of ill-defined upper respiratory infections such as the common cold which accounted for most absences.

5. Miscellaneous Applications

UV is often employed to maintain sterile conditions during packaging of pharmaceutical and biological products. One concern has reported the successful use of a special hood into which UV treated air is passed (156). A sketch of this hood is shown in Figure 35. A number of modifications of this hood are in use in other pharmaceutical houses at the present time.

Matelsky (202) has reported the use of UV radiation by a pharmaceutical products company to prevent the spread of infectious agents from experimental animals to workers.

Ultraviolet radiation has been used in the United States and Canada by sugar refineries to eliminate thermophilic bacteria and yeasts present on sugar crystals (27,113). Ultraviolet lamps also are used to prevent mold growth on liquid sugar in the storage tanks.

Nelson and Matelsky (222) reported good results with the use of UV radiation in a cherry packaging plant. The placement of lamps in vital spots reduced the total bacterial air count, eliminated Mycoderm scum on processed cherries and eliminated mold growth on the surface of casks.

Coblentz (45) reported in 1942 on the testing of an artificial UV applicator designed for use by the medical profession for irradiating the fundus of deep cavities. He concluded that the applicator was a dangerous instrument unless the intensities received by the patient in the deep cavities were rigidly controlled.

The use of UV radiation has been suggested many times for the pasteurization of milk. As yet, no proved method has been developed for efficient, large scale treatment of such liquids. Most suggested methods

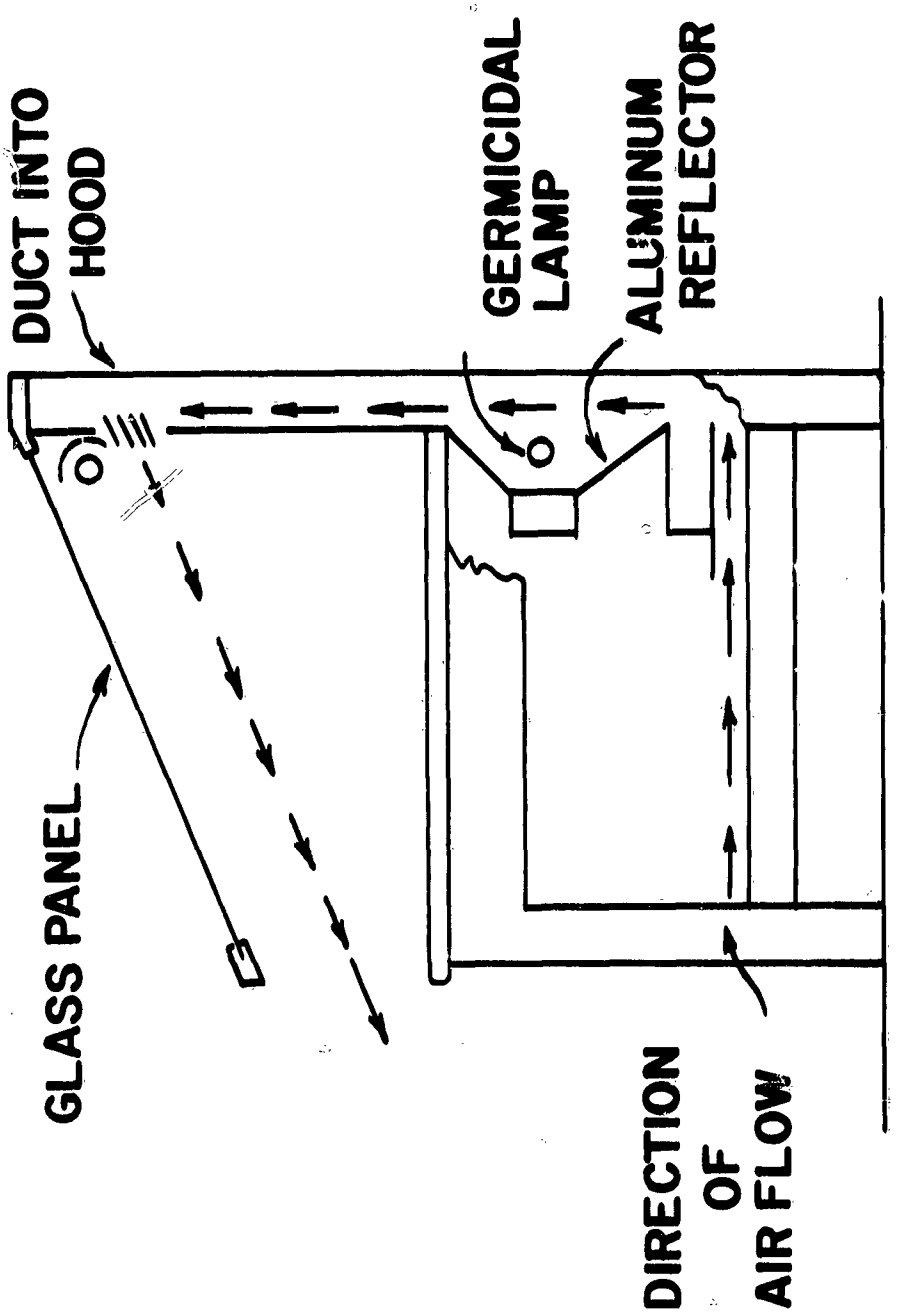


Figure 35. Product Production with UV Radiation.

have involved the exposure of a thin film of the liquid to high intensity UV radiation. Nicholson (224) in 1947, designed a small pasteurizing unit for milk which he recommended for use on small dairy farms. The apparatus was reputed to produce milk with bacterial counts of less than 2500 organisms per milliliter. The milk was exposed to UV radiation as it flowed over rotating cylinders. By controlling the speed of the cylinders, the thickness of the film of milk could be adjusted. A fan was used to remove the ozone. Radiation was supplied by a battery of eight, 15-watt hot cathode lamps. In addition to the low bacterial count, it was claimed that the flavor of the milk was improved.

Nagy (220) reported on a method for the sterilization of opaque liquid by passing the liquid through long sections of small diameter Vycor tubes with the high intensity lamps on the outside of these tubes. In this manner the liquid would not be exposed to ozone or other gases. Rapid movement of the liquid in the tubes would greatly increase the turbulence so that all of the liquid would be exposed to the radiation. Milk exposed in this manner acquired an undesirable flavor. It was believed that the 2537A radiation altered some of the sulphur containing proteins. The "off" flavor was a result of this photochemical reaction rather than oxidation by ozone.

Laboratory devices using UV radiation have been used as a means of sterilizing sugar solutions, culture media, and tissue. One such apparatus was reported by Carlson et al (43). A small aluminum lined box containing an 8-watt germicidal lamp was used. Processed X-ray film was used over the top of the box to prevent the radiations from reaching the eyes. Liquids were sterilized in small quartz flasks. Advantages claimed for this method of sterilization were (a) no loss of water, (b) no heat-induced chemical reactions, (c) solutions may be prepared with unsterile pipettes, and (d) time saving; exposure times as short as five minutes were sufficient. The primary disadvantage listed was that prolonged exposure to UV radiation may produce chemical changes in some substances.

A device called the "Baryaire" has been manufactured by Hanovia Chemical and Manufacturing Company. It was recommended that these units be placed in hospital corridors to prevent cross-infection by isolating certain areas. A blower was utilized to draw the air over the UV lamp. Magondeaux (199) claimed that air-borne microorganisms could not be carried past the UV barrier. A sketch of this unit is shown in Figure 36.

Dr. M. B. Lurie of the University of Pennsylvania has reported over a period of years on air-borne transmission of tuberculosis and its control by UV radiation. These studies contain many valuable data applicable to the problem of safe storage and handling of infected animals. Lurie (194) demonstrated that air is a natural vehicle of the contagion of tuberculosis in animals and that normal rabbits and guinea pigs acquired pulmonary tuberculosis when placed in individual open cages in a room housing animals infected with tubercle bacilli.

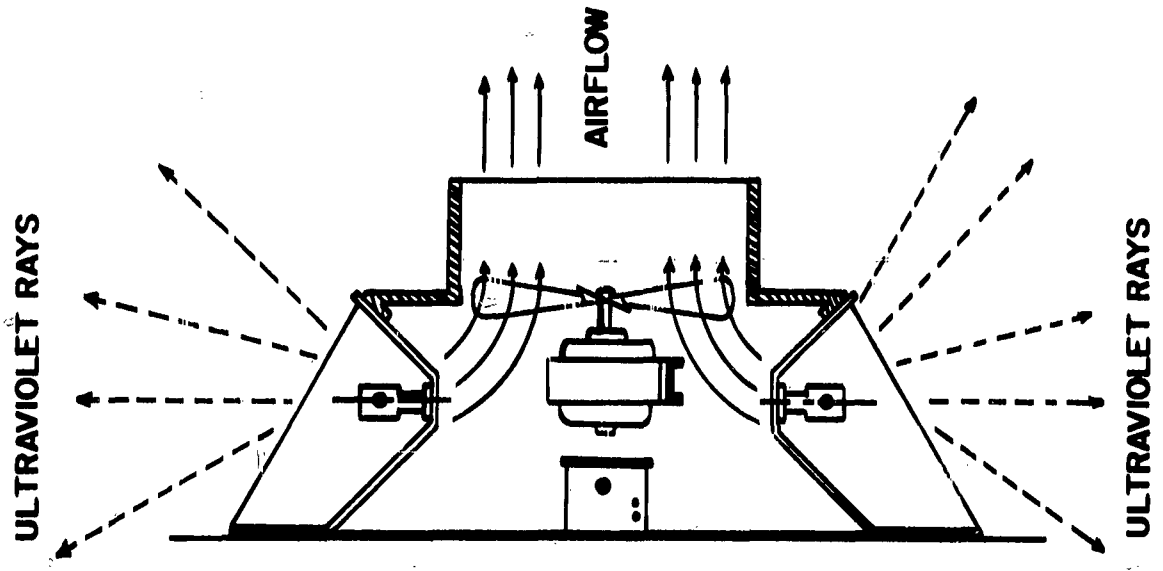


Figure 36. Baryaire Safe-T-Aire Equipment.

Other publications described the use of UV radiation to prevent the spread of air-borne infection of tuberculosis in animals (195-197,308). Two identical animal rooms were used. Each room had two cage racks for housing rabbits placed several inches apart. Infected animals were placed in one cage rack and uninfected rabbits in the other. One room was equipped with UV radiation with the lamps placed horizontally in the space between the two cage racks at each shelf level radiating downward. Also, UV lamps were placed above and below the cage racks. The experiment was carried on over a period of one year during which time 11 of the 15 uninfected rabbits in the unirradiated rooms died of tuberculosis. Three of the remaining rabbits developed a "regressive microscopic tubercle" and none developed tuberculin sensitivity. None of the control rabbits in the irradiated room contracted tuberculosis. "Thus a 73 per cent mortality from tuberculosis was eliminated by UV radiation."

The application of germicidal UV radiation falls logically into two categories, product protection and personnel protection. The majority of the UV lamps in use today are for purposes of product protection, although new research in the field of public health, laboratory hazards, etc., will probably increase the use of germicidal energy for personnel protection. Below are listed a few of the applications of UV radiation and certain industries in which germicidal lamps are commonly utilized:

Applications for Germicidal UV Radiations

(1) Preparation and packaging pharmaceutical products
(2) Sanitation of drinking glasses, plates and cutlery
(3) Sanitation of walls and fixtures in bathrooms
(4) Killing thermophilic bacteria in sugar
(5) Vaccine production
(6) In air-conditioning units
(7) Hospital nursery rooms
(8) Operating rooms
(9) Dairies
(10) Bottling plants
(11) Preparation of blood plasma
(12) Poultry and animal protection
(13) Protection of baked goods from mold
(14) Protection of meat from mold and bacteria
(15) Tenderization of meat
(16) Cold storage of fruits and vegetables
(17) Sterilization of water
(18) Schools
(19) Aging of cheese
(20) Breweries
(21) Sterilization of supply or exhaust air
(22) Preparation of wine

UV radiations can be used in infectious disease laboratories for personnel protection as well as for product protection. In the literature reviewed, no comprehensive study of practical types of installations for personnel protection could be found except those studies dealing with the use of UV in air-conditioning systems. Various types of barriers and locks have been reported but design details and adequate evaluation tests were lacking.

A comprehensive experimental program directed toward the design and evaluation of specific UV installations, suitable for use in infectious disease laboratories has been conducted by the authors over a period of approximately eight years. A detailed account of some of these studies is presented on the following pages.

B. AIR LOCKS

An air lock is defined as a small empty room with a door at each end, constructed to create a dead air space for a safer passageway between two areas. Germicidal lamps are installed on the ceiling of such rooms. Experiments were conducted to determine the effectiveness of UV radiation in preventing the passage of air-borne microorganisms from area to area.

Cultures of S. marcescens were used in most of these studies. In some tests, normal bacterial flora of the air or surface contaminants were used as indicators of germicidal effectiveness. Aerosols of S. indica or S. marcescens were produced from 24-hour broth cultures by a DeVilbiss No. 40 nebulizer.

To evaluate the effectiveness of UV air locks, air was sampled for bacterial content by sieve-type air samplers (Figure 37) with the UV lamps off and on. In some instances liquid impinger samplers were used for the UV off air samples. The comparative number of organisms recovered and the per cent reduction allowed an estimation of the effectiveness of the germicidal radiation. Some tests were done to evaluate the action of the radiation on surfaces in an air lock.

During these studies some attention was given to the phenomenon of photoreactivation, first described by Kelner (169). Recovery plates were sometimes prepared in duplicate and incubated under white light and in the dark. However, our experiments called for lethal concentrations of radiation and were performed during the day when generous amounts of white light were present before and during the tests, and no photoreactivation was demonstrated.

1. Air Lock for Field Change Room

The effectiveness of an UV installation used during a series of summer field tests was determined. The installation consisted of an UV air lock room through which contaminated personnel returning from field tests passed before entering a contaminated change room. The bacteriological studies conducted in this UV barrier are grouped under three headings:

(a) Killing of air-borne clouds passing through the air lock,

(b) decontamination of surfaces, and

(c) decontamination of protective field clothing.

The floor plan of the lock is shown in Figure 38. Nine 36-inch, 30-watt hot cathode UV lamps were installed in the lock. Eight of the lamps were located vertically on the side walls while the remaining lamp was in the ceiling at a distance of about eight feet from the floor. None of the lamps were equipped with reflectors. Before tests were conducted the lamps were carefully cleaned with 95 per cent ethyl alcohol and their output measured.

The test organism used in these studies was S. indica. Recovery of the test organism was accomplished with cotton swabs and sieve-type air samplers. The plating medium in both cases was Difco's Tryptose Agar. All agar plates were incubated for 48 hours at room temperature.

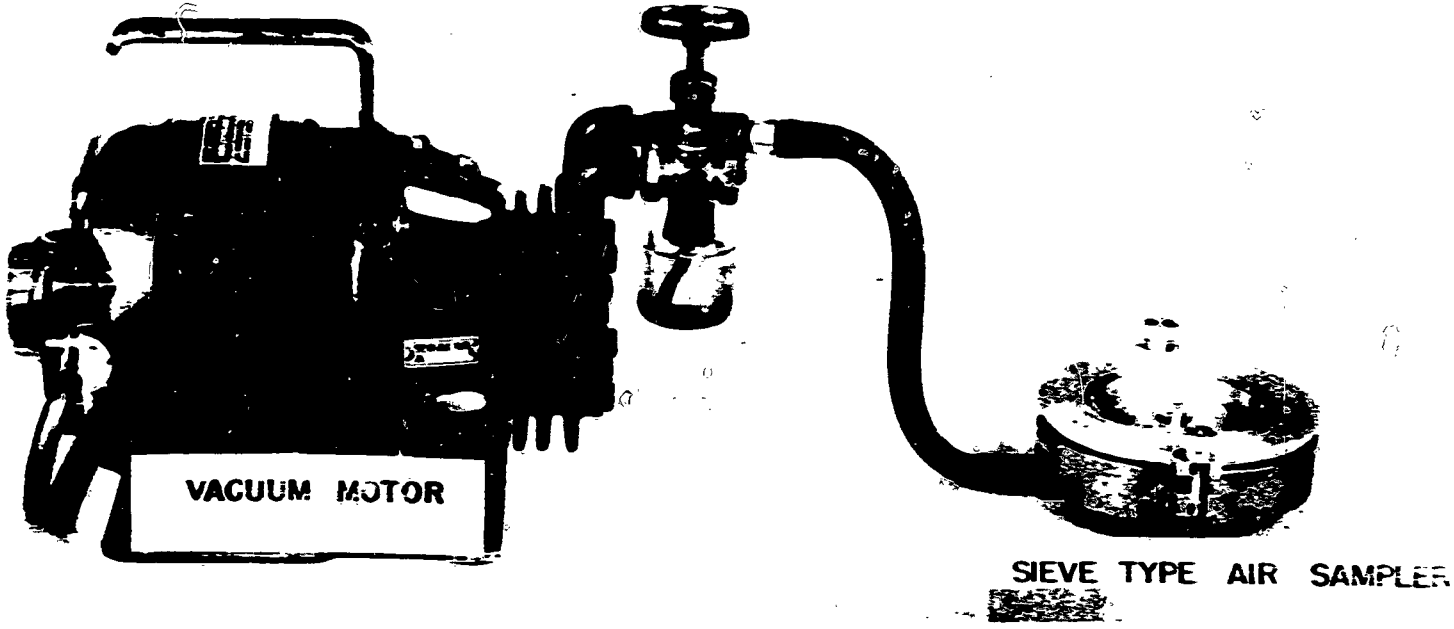


Figure 37. Sieve-Type Air Sampler. (FD Neg C-5827)

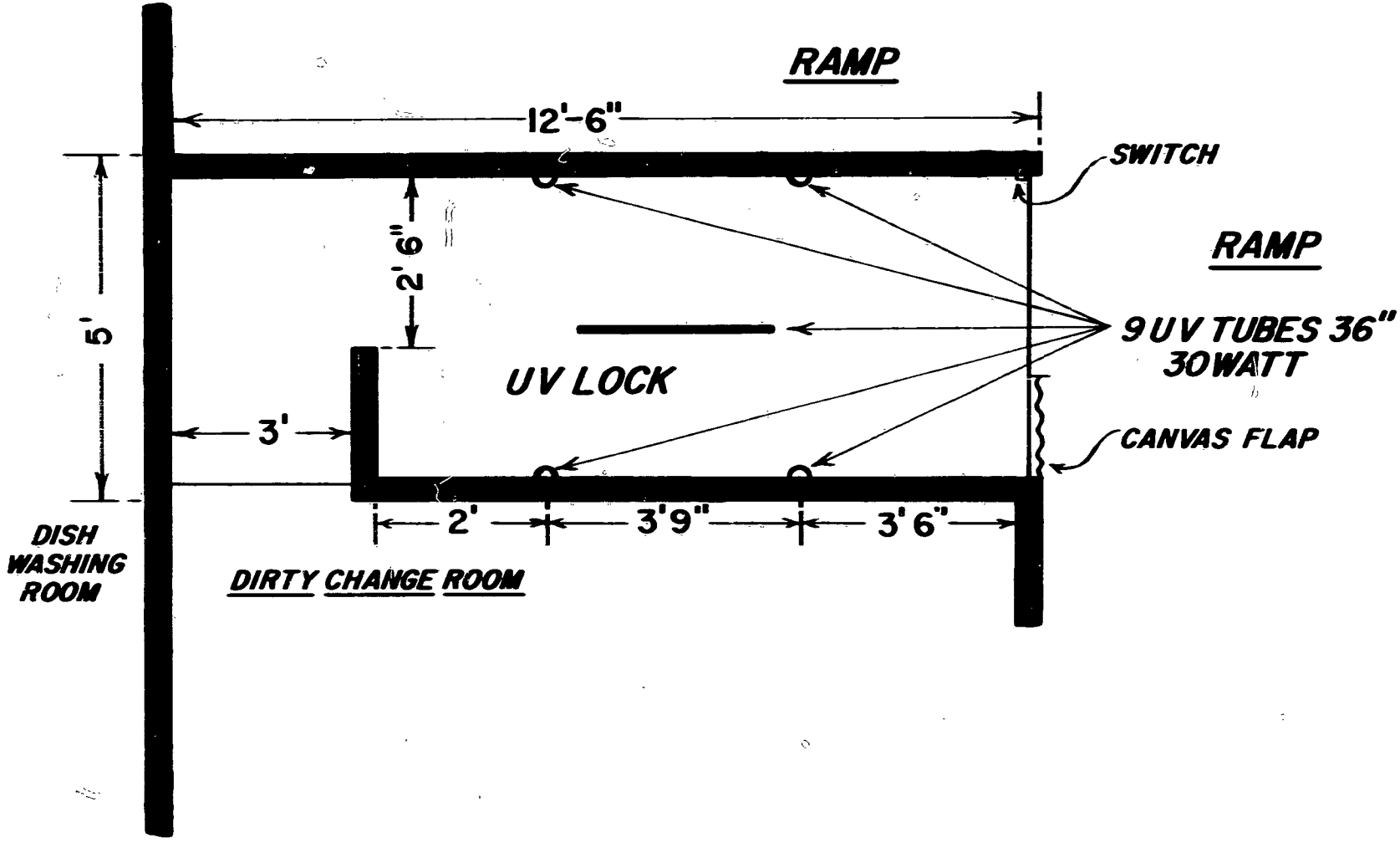


Figure 38. UV Entrance Lock.

a. Inactivation of Air-Borne Clouds Passing Through the UV Air Lock

The UV air lock led to the contaminated change room. The change room was equipped with an exhaust air blower and filter unit. With the exhaust air blower in operation, a constant flow of air was maintained through the UV barrier in the direction of the change room.

Nebulizers were set up at the outside opening of the UV lock, two feet from the floor. Air samples were taken at the same height in the doorway leading to the contaminated change room. Control air samples were taken before each test. During the tests, the bacterial cloud was generated continuously. Five-minute air samples were taken first with the UV lamps burning and then with them off. This procedure was continued for several successive off and on periods. The results of the two tests (Table XXXIII) show a marked reduction of viable air-borne organisms obtained by the treatment with the UV radiation. The initial cloud concentration passing through the lock was estimated at approximately 1×10^9 air-borne S. indica cells per minute. It will be noticed that the number of colonies recovered when the lamps were on increased as each test proceeded. This is due to incomplete removal, by the exhaust system, of the cloud which passed into the dressing room during the time when the lamps were off.

TABLE XXXIII. INACTIVATION OF AIR-BORNE S. INDICA IN A FIELD AIR LOCK

OPERATION	COLONIES ON 5-MINUTE AIR SAMPLES	
	Test 1	Test 2
UV on	4	2
UV off	TNTC	TNTC
UV on	26	250
UV off	TNTC	TNTC
UV on	300	300
UV off	TNTC	TNTC

Because the concentration of air-borne bacteria in these tests far exceeds the maximum number that would be expected during actual use of the lock, it is apparent that a considerable amount of protection is afforded by this UV installation.

b. Decontamination of Surfaces

The four surfaces used in these tests were plywood, glass, rubber, and canvas. Duplicate sections of each surface were obtained. One section served as control and the other was exposed to the radiations in the UV lock. The surfaces were contaminated by holding them for several minutes a short distance in front of three DeVilbiss No. 40 nebulizers. All surfaces were contaminated in a like manner. The control surfaces were placed outside in the shade and the test surfaces were placed on the floor in the center of the UV air lock. The lamps in the lock were turned on and samples taken with cotton swabs at five-, ten-, and fifteen-minute intervals. The swabs were streaked onto Difco's Tryptose Agar plates and incubated for 48 hours at room temperature. Except for one colony recovered from the glass surface after the five-minute exposure period, no S. indica was recovered on any of the surfaces exposed to the UV radiation. The results of the tests are shown in Table XXXIV. Viable organisms were obtained from all control samples throughout the test. These data show that any organisms that may be shaken off onto the floor or walls of the lock will be inactivated unless protected from the UV radiations.

TABLE XXXIV. INACTIVATION OF S. INDICA ON SURFACES IN A FIELD AIR LOCK

SURFACE	NUMBER OF S. INDICA COLONIES					
	Exposure Time					
	5 Minutes		10 Minutes		15 Minutes	
	Control	UV	Control	UV	Control	UV
Plywood	12	0	7	0	3	0
Glass	TNTC	1	TNTC	0	TNTC	0
Rubber	TNTC	0	TNTC	0	33	0
Canvas	TNTC	0	TNTC	0	TNTC	0

c. Decontamination of Personnel Wearing Protective Field Clothing

In each of these tests two persons wearing protective field clothing and assault-type gas masks were exposed to a heavy cloud of S. indica organisms for a period of time in a closed room. The aerosol was generated by three DeVilbiss No. 40 nebulizers which were placed in the room. For the first test, the persons remained in the room for a period of 15 minutes, and for the second test the exposure time was 30 minutes.

After the artificial contamination, the test proceeded as follows: Control surface samples were taken at seven locations on the suit and mask of each individual. One individual entered and walked about in the UV lock with the lamps on, while the other individual (the control) remained outside in the shade. After selected intervals of exposure, the test individual left the lock, and samples were taken from seven locations on the suit and mask of each individual. The test individual then re-entered the UV lock and the process was repeated. The sampling locations were: Top of hood, right arm, left arm, right leg, left leg, chest, and back. The surface samples were streaked onto Difco's Tryptose Agar plates, the plates incubated for 48 hours at room temperature and colonies of S. indica counted.

Tables XXXV and XXXVI show the efficiency of the UV radiation in inactivating the bacteria present on the protective clothing and masks of the test individual. All of the areas sampled were those exposed to the UV radiation. No swabs were taken of protective areas such as under the arms, as no germicidal action would be expected in these areas.

An interesting observation was made during these tests. Numerous organisms (mostly spore forming bacteria and yeasts) were present on the surfaces of the protective clothing, assault masks, and in the air in the lock. These were recovered in significant number on the control surface and air samples. It was observed that as each test proceeded the number of these contaminants on the recovery plates became less. The samples taken during the last sampling period were sterile and no growth of the contaminants was noted.

These tests conducted on an UV barrier at the entrance to a field dressing room show the germicidal barrier to be efficient in inactivating test organisms on exposed surfaces, on protective clothing and in the air.

2. Laboratory Air Lock Number 1

Three 30-watt UV lamps were installed on the ceiling in an air lock 8 feet long, 3½ feet wide and 10 feet high (Figure 39). Movement of air between the rooms separated by this air lock was controlled during testing by means of exhaust fans, although in practice the room of greater infectious hazard is kept at a negative pressure.

A meter employing a WL-775 Tantalum photocell and calibrated for response at wave length 2537A was used to determine the radiant intensities of energy throughout the air lock. All measurements were taken on a horizontal plane, and the radiation measured represented energy received from above. With the exception of one reading, all areas received at least 30 microwatts per square centimeter (Table XXXVII).

TABLE XXXV. DECONTAMINATION OF PERSONNEL IN A FIELD AIR LOCK, ONE TO FIVE MINUTES' EXPOSURES[a]

SWAB POSITION	NUMBER OF S. INDICA COLONIES					
	Test Individual			Control Individual		
	Exposure Time to UV			Exposure Time in Shade		
	1 min	2 min	5 min	1 min	2 min	5 min
Top of hood	1	0	0	TNTC	TNTC	TNTC
Right arm	3	0	0	TNTC	TNTC	TNTC
Left arm	2	0	0	TNTC	TNTC	TNTC
Right leg	2	0	0	TNTC	TNTC	TNTC
Left leg	4	0	0	TNTC	TNTC	TNTC
Chest	10	2	0	TNTC	TNTC	TNTC
Back	4	1	1	TNTC	TNTC	TNTC

a. Individuals were exposed to the aerosol for 15 minutes.

TABLE XXXVI. DECONTAMINATION OF PERSONNEL IN A FIELD AIR LOCK, FIVE TO TWENTY MINUTES' EXPOSURES[a]

SWAB POSITION	NUMBER OF S. INDICA COLONIES							
	Test Individual				Control Individual			
	Exposure Time to UV				Exposure Time in Shade			
	5 min	10 min	15 min	20 min	5 min	10 min	15 min	20 min
Top of hood	0	0	0	0	TNTC	TNTC	TNTC	TNTC
Right arm	1	0	0	0	TNTC	TNTC	TNTC	TNTC
Left arm	0	0	0	0	TNTC	TNTC	TNTC	TNTC
Right leg	2	0	0	0	TNTC	TNTC	TNTC	TNTC
Left leg	0	0	0	0	TNTC	TNTC	TNTC	TNTC
Chest	16	0	0	0	TNTC	TNTC	TNTC	TNTC
Back	9	0	0	0	TNTC	TNTC	TNTC	TNTC

a. Individuals were exposed to the aerosol for 30 minutes.

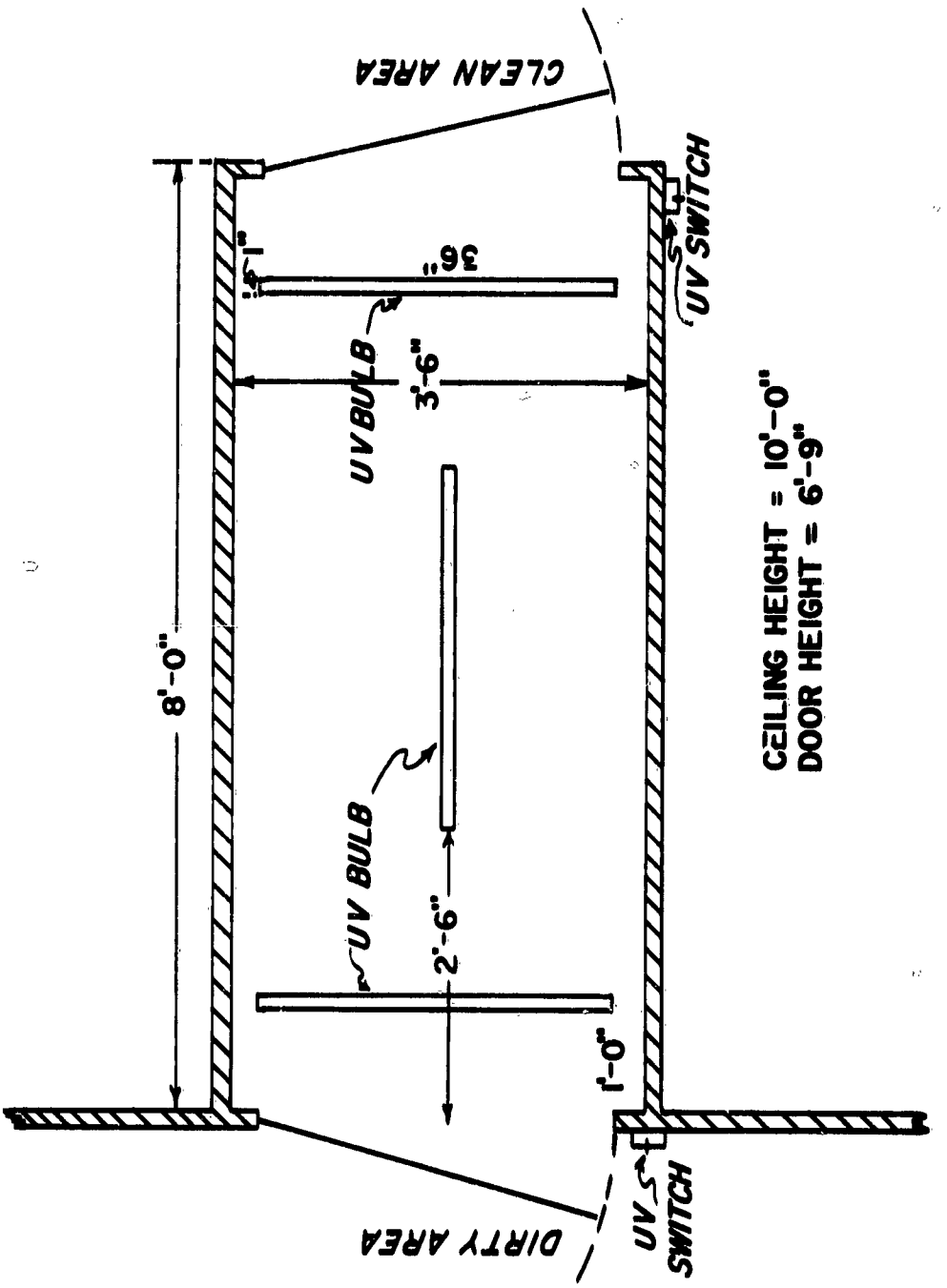


Figure 39. UV Air Lock.

TABLE XXXVII. UV INTENSITIES IN AN 8' x 3½' x 10' AIR LOCK EQUIPPED WITH THREE 30-WATT LAMPS

DISTANCE FROM FLOOR LEVEL, inches	MICROWATTS PER SQUARE CENTIMETER						
	Distance in Feet from North to South End						
	1	2	3	4	3	2	1
8	10	38	43	44	41	40	33
24	33	43	44	49	50	48	46
40	52	56	57	59	59	59	54
60	81	81	80	75	75	74	85
90 (30 inches below ceiling)	157	118	115	147	112	110	144

Bacteriological tests were conducted with the doors open and closed. Aerosols of S. indica were produced outside the air lock on the upwind side, and samples were taken outside the air lock on the downwind side. The bacterial aerosol concentration was controlled by nebulizing a culture that had been diluted to the desired concentration. Generation of the aerosol continued throughout each test. The results show at least a 99-per cent reduction of the bacterial aerosol in every case (Table XXXVIII).

In one case complete removal of the aerosol was accomplished. The efficiency of the UV air lock under the stated test conditions was of a high order.

In test four, the direction of the flow of air was reversed and the doors of the lock were closed. Reverse movement of air was effected by operating the two wall exhaust units located on the clean side of the lock. The aerosol in this case was generated in an adjacent room. The door leading from this room to the rest of the building was, at this time, open. Even though there was a slight negative pressure in the main hallway at the time of the test, large numbers of the test organisms were pulled through the lock into the clean area. However, operating the UV lamps under these conditions resulted in a 99 per cent removal of the test organism.

3. Laboratory Air Lock Number 2

Three tests were conducted to determine the bactericidal effectiveness of an UV air lock seven feet long installed in the hallway separating two buildings. A small glass window was removed from one of the doors on one side of the lock to allow the movement of air from the first building through the lock and into the other building at the rate of approximately two linear feet per second.

TABLE XXXVIII. BACTERIOLOGICAL TESTS OF AN UV AIR LOCK USING S. INDICA AS THE TEST ORGANISM

TEST NUMBER	AIR VELOCITY, Feet Per Second	POSITION OF AIR LOCK DOORS	CLOUD CONCENTRATION PER CUBIC FOOT OF AIR			PER CENT REDUCTION OF S. INDICA
			At Nebulizing Position	At Collection Point Past the Air Lock		
				UV On	UV Off	
1	2	open	408	0.4	43	99
2	2	open	938	0	81	100
3	2	open	3,347	0.8	334	99
4	---a/	closed	110,600	14	11,600b/	99

a. Leakage around door. b. Estimated.

NOTE: No S. indica appeared in control air samples taken before each test. Organisms passing through the air lock were collected with sieve samplers for five minutes at one cubic foot per minute. Liquid impingers were used to determine the number of organisms per cubic foot of air at the point of nebulization. The collection efficiency of the samplers is estimated at 95 per cent for the liquid impinger and 45 to 70 per cent for the sieve sampler.

Radiation in the lock was supplied by two Slimline lamps. These units were mounted horizontally on the side walls of the lock 12 inches below the ceiling, and each lamp was equipped with a reflector.

Using a meter with a WL-775 phototube, intensity measurements were taken in the lock. Readings were made at measured distances from the ends of the lock and from the floor level (Table XXXIX). Low concentrations of UV radiation were at the floor level and in the corners of the lock at upper levels. Because of the reflector system high intensity UV radiation was directed across the upper part of the chamber with lesser amounts of energy reaching the floor and corners of the room. S. indica culture was diluted in physiological saline and nebulized on the positive pressure side of the air lock with a DeVilbiss number 40 glass nebulizer. The diluted culture was dispersed at the rate of approximately 0.7 milliliter per minute. For the three tests conducted, three different dilutions of the S. indica were used.

TABLE XXXIX. UV INTENSITIES IN AIR LOCK NUMBER 2

DISTANCE FROM FLOOR, feet	INTENSITIES IN MICROWATTS PER SQUARE CENTIMETER		
	East End	Middle	West End
	Through Center of Air Lock		
1	13.6	26.6	7.4
3	8.0	18.0	6.4
5	2.0	3.6	2.0
7	2.0	2.0	2.0
	Side of Air Lock		
1	2.0	4.0	3.6
3	2.0	3.0	2.0
5	2.0	49.2	6.0
7	14.8	14.8	4.0

Note: Measurements were made on a horizontal plane of radiation received from above. Intensities at the 3- to 5-feet level of side radiation were on the order of 100 to 150 microwatts per square centimeter.

Nebulization of the test organism was continued at a constant rate during each experiment. Liquid impinger air samples were taken on the positive pressure side of the lock to establish the aerosol concentration. Air samples were taken with two sieve-type samplers on the low pressure side of the air lock. One sampler was placed at a distance of two feet in front of each door, and two feet above the floor level. The samplers contained plates of corn steep molasses agar. The rate of sampling was one cubic foot of air per minute, and the duration of each sample was five minutes. Each test was divided into three parts as follows:

(a) Samples taken on the negative pressure side of the air lock before nebulization started (control samples).

(b) Samples taken on the negative pressure side of the air lock during the first five minutes of nebulization. UV lamps on.

(c) Samples taken on the negative pressure side of the air lock during the second five minutes of nebulization. UV lamps off.

The results obtained from the three tests are summarized in Table XL. A high reduction of organisms was obtained under the stated test conditions. The over-all average reduction of viable S. indica cells was 97 per cent. Aerosol concentrations ranging from 33 to 3,883 organisms per cubic foot were used.

TABLE XL. RESULTS OF BACTERIOLOGICAL TESTS IN AN UV AIR LOCK

TEST CONDITION	TOTAL NUMBER OF S. INDICA COLONIES RECOVERED FROM TWO SIEVE SAMPLERS IN FINE MINUTES		
	Test 1	Test 2	Test 3
Control	0	0	0
UV on	0	12	123
UV off	108	554	1940[a]
Cloud conc at nebulizing position, org per cu ft	33	280	3883
Percentage reduction of cloud with UV on	100	98	94

a. This figure was obtained by calculating the percentage of the generated cloud which passed through the lock during the first two tests when the UV was off. The actual number of colonies on the sampler plates were too numerous to count.

The over-all average per cent reduction for the three tests was 97.

4. Laboratory Air Lock Number 3

Tests were conducted to determine the bactericidal effectiveness of an UV lock 24 feet long, seven feet wide, and ten feet high. There were three doors leading into the air lock, and three bare 30-watt hot cathode germicidal lamps were installed in the ceiling.

Studies were made, using smoke, to determine the rate and direction of movement of air through the lock. The direction of air movement was toward the more contaminated building. The rate of movement was slow, estimated to be one-half foot per second. There was a considerable degree of turbulence within the lock, and smoke entering one end of the lock was well mixed by the time it reached the outlet end.

New, clean lamps were installed in the three ceiling fixtures and intensity measurements were made as shown in Table XLI (A). Readings were also made of the radiant energy flux directly below the center lamp while all lamps were burning. These results are also shown in Table XLI (B).

TABLE XLI. UV INTENSITIES[a] IN AIR LOCK NUMBER 3

A.

DISTANCE FROM EAST END OF AIR LOCK	DISTANCE ABOVE FLOOR LEVEL		
	8 inches	3 feet	5 feet
0	8.4	5.8	5.8
3 feet	19.9	19.2	45.1
6 feet	13.2	19.2	29.2
9 feet	18.9	9.2	8.0
12 feet	20.5	41.6	81.3
15 feet	14.4	11.5	15.4
18 feet	23.0	23.1	19.2
21 feet	11.8	28.5	72.0
Averages	16.3	19.8	34.5

B.

DISTANCE ABOVE FLOOR LEVEL	MICROWATTS PER SQ CM
8 inches	23.4
2 feet	31.2
4 feet	49.2
5 feet	71.1
6 feet	112.0
Average	57.4

a. Microwatts per square centimeter.

The bacteriological tests were conducted in the usual manner. S. indica culture was nebulized in the doorway on the upwind side and the sieve air samplers were operated in the room on the downwind side. Control air samples taken before nebulization started were negative. The results are shown in Table XLII.

TABLE XLII. RESULTS OF BACTERIOLOGICAL TESTS IN AN UV AIR LOCK

TEST CONDITION	NUMBER OF S. INDICA COLONIES RECOVERED FROM TWO SIEVE SAMPLERS IN FIVE MINUTES	
	Test 1	Test 2
Control	0	0
UV on	0	2
UV off	303	800 approx.
UV on	9	3
UV off	146	800 approx.
Cloud concentration, orgs per cu ft	180	790
Over-all per cent efficiency	98	99

In spite of the low UV intensities present, excellent lethal effects on the air-borne S. indica were obtained. The UV air lock was 98 to 99 per cent effective in inactivating the test organisms. These results indicate the importance of air velocity and air lock size. Both larger exposure areas and slower rates of air movement increase the exposure time of air-borne organisms passing through the lock.

Our experience with a variety of similarly radiated air locks has shown that few, if any, air-borne vegetative bacteria or bacteriophage particles will penetrate such a barrier if air velocities of about two feet per second or below are involved and if the number of UV lamps attached to the ceiling provide a floor intensity of 20 to 30 microwatts per square centimeter.

C. DOOR BARRIERS

In the absence of an air lock, an effective barrier can be made by providing a radiation screen across a doorway. A design we recommend for

this purpose uses five 17-watt cold cathode UV lamps with aluminum reflectors placed in a wood or metal channel built around the doorway (Figure 40). The channel is placed so that the door opens away from the barrier. In this manner a screen of high-intensity ultraviolet radiation is projected across the doorway.

Tests on an UV door barrier are presented on the following pages. The experimental methods were essentially the same as those used in testing UV air locks. The door leading from the main hallway of a laboratory building into a clean waiting room was equipped with an UV barrier consisting of five 30-watt, 36-inch, hot cathode germicidal lamps. The air system in the hallway was such that a negative pressure was present. Before beginning the tests, all lamps were carefully cleaned with ethyl alcohol.

Intensity measurements were taken within the UV barrier using a meter with a WL-776 phototube. All readings were expressed in terms of microwatts per square centimeter. Table XLIII shows the results obtained from these readings. It is apparent that the intensities obtained in the barrier are quite high. Because lamps of this type drop in UV output the first few hours of operation, a more accurate estimate of the intensities to be encountered may be obtained by reducing each value in Table XLIII by 10 per cent. S. indica culture was diluted in physiological saline and nebulized with a DeVilbiss number 40 glass nebulizer. Three dilutions of the S. indica culture were used with a dispersal rate of approximately 0.5 milliliter per minute. Nebulization was continued at a constant rate during each experiment. Liquid impinger air samples were taken to establish the initial aerosol concentration. Four tests were made with this door barrier.

TABLE XLIII. UV INTENSITIES IN MICROWATTS PER SQ CM AT THE VERTICAL CENTER OF A DOOR BARRIER

FEET ABOVE THE FLOOR	RADIATION FROM ABOVE	RADIATION FROM LEFT	RADIATION FROM RIGHT	TOTAL ENERGY FLUX RECEIVED
6	166	142	144	452
5	96	126	126	348
4	66	113	110	289
3	52	126	144	322
2	36	96	120	252
½	28	40	30	98

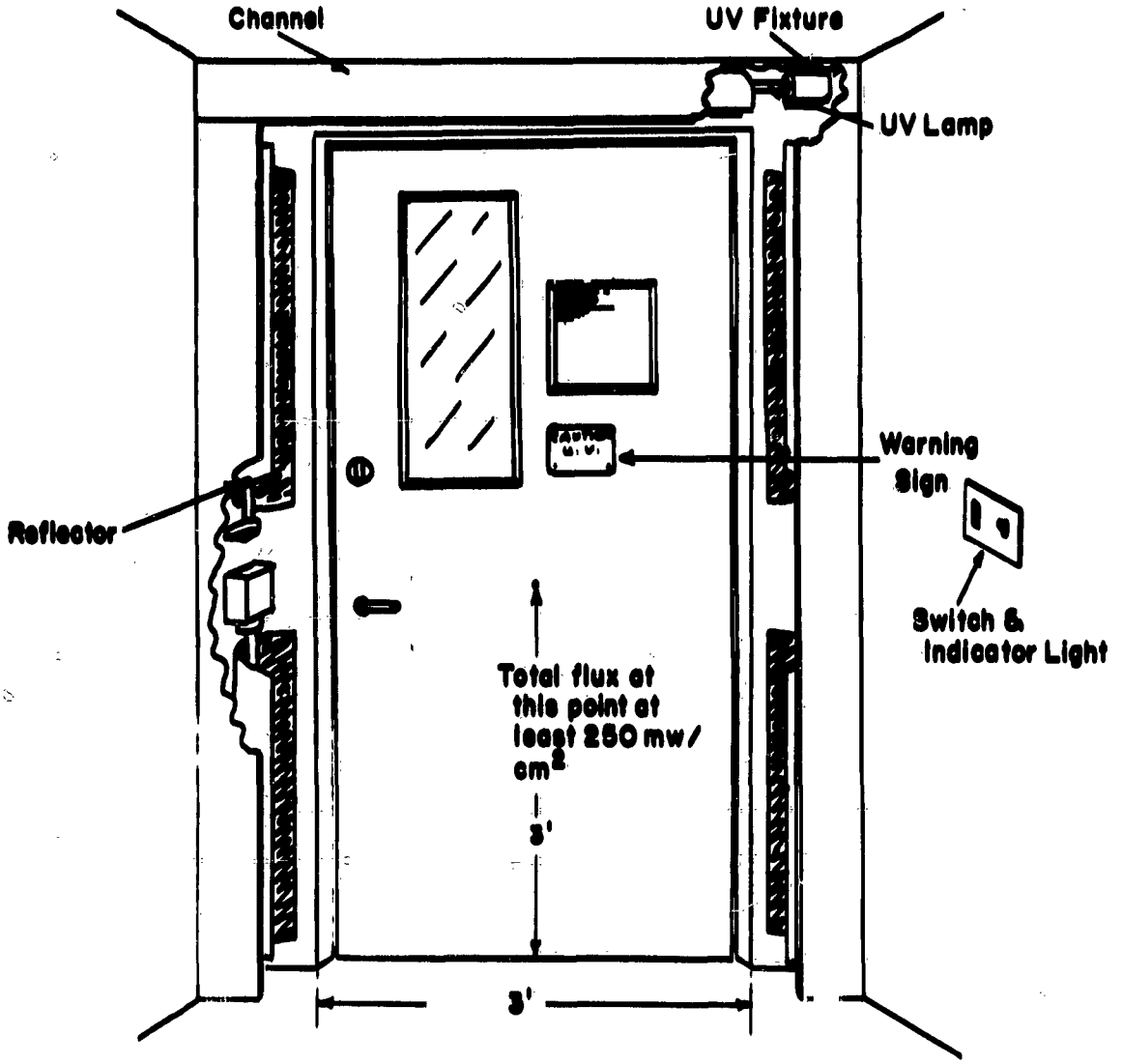


Figure 40. UV Lamp Installation at Doorway.

Test 1: An aerosol containing 40 organisms per cubic foot was generated in the entrance waiting room which was on the positive pressure side of the UV door barrier. All doors remained closed during the test, and air samples were taken with two sieve samplers on the negative pressure side (main hallway). Control air samples taken before the aerosol was generated were negative. The results are shown below:

Test Conditions	Number of S. indica Colonies Recovered from Two Sieve Samplers in 5 Minutes
Control	0
UV on	0
UV off	27

The efficiency of the UV barrier was 100 per cent under these conditions.

Test 2: S. indica aerosol was generated on the negative pressure side of the door barrier, and the sieve samplers were located on the positive pressure side (small waiting room). The aerosol concentration was 10,800 cells per cubic foot. All doors were closed during this test. The cloud was generated continuously for 20 minutes and two sets of air samples were taken with the UV lamps on and two sets with the UV lamps off.

No S. indica organisms were collected on the positive pressure side of the UV barrier. This test shows that air-borne organisms in the main hallway of the building will not escape into the waiting room when the conditions are static (no movement of personnel or opening of doors).

Test 3: By using smoke it was found that the pumping action of a door on the positive side of the door barrier momentarily upset the air balance in the building and drew smoke through the door barrier to the positive pressure side from the negative pressure area. This test was designed to determine the efficiency of the UV radiation in preventing "back flow" of airborne organisms when the outside door is opened.

An aerosol containing 214,000 particles per cubic foot was generated in the main hallway. Air samples were taken in the waiting room. Throughout the test, at intervals of one-half minute, the outside door was opened. Control air samples taken before the aerosol was generated were negative. The results are shown below:

Test Conditions	Number of S. indica Colonies Recovered from Two Sieve Samplers in 5 Minutes
Control	0
UV on (outside door opened 10 times)	1
UV off (outside door opened 10 times)	291

The efficiency of the UV door barrier under these conditions was greater than 99 per cent.

Test 4: This test was designed to determine the efficiency of the UV door barrier in preventing the escape of air-borne organisms from within the building when entrances and exits were made through the barrier door. At intervals of two and one-half minutes, a person made an entrance into and an exit from the main hallway through the barrier door. The generated cloud in the main hallway contained 214,000 organisms per cubic foot. Samples were taken in the waiting room. Control air samples taken before the aerosol was generated were negative. The results obtained are shown below:

Test Conditions	Number of S. indica Colonies Recovered from Two Sieve Samplers in 5 Minutes
Control	0
UV on (two entrances and two exits)	67
UV off (two entrances and two exits)	889

The efficiency of the UV door barrier under these test conditions was 92.5 per cent.

Summary Table XLIV presents the test conditions and the respective efficiencies of the UV door barrier. The efficiencies of the UV door barrier under the stated test conditions were very high. In most cases the cloud concentrations were many times that which would be expected under normal conditions. Efficiencies varying from 92.5 per cent to 100 per cent were obtained under the stated test conditions.

Curtains made of strips of plastic sheeting provide a convenient method of limiting stray radiation from a door barrier. Figure 41 shows a curtain made of clear vinyl plastic installed in front of a door barrier.

D. ULTRAVIOLET ANIMAL CAGE RACKS

Surveys of laboratory acquired infections (278), studies on cross-infections among animals (194,237,238) and institutional outbreaks with infectious agents (48,141,205,253), have demonstrated the need for adequate separation of infectious animals from laboratory workers and from other animals. Although the best isolation is obtained by the use of closed cages equipped with filters and artificially ventilated (59,155,300), in many laboratories the expense of this equipment is prohibitive.

TABLE XLIV. BACTERIOLOGICAL TESTS OF AN UV DOOR BARRIER

TEST CONDITIONS	POINT OF NEBULIZATION OF *S. INDICA*	POSITION OF SIEVE SAMPLERS	CLOUD CONCENTRATION PER CUBIC FOOT OF AIR			PER CENT EFFICIENCY OF UV DOOR BARRIER
			At Nebulizing Point	At Collection Point by Sieve Samplers		
				UV on	UV off	
Hall door closed	Positive pressure side	Negative pressure side	40	0	5.4	100
Outside door opened 10 times	Negative pressure side	Positive pressure side	214,000	0.2	58	99.7
Two entrances and two exits by man	Negative pressure side	Positive pressure side	214,000	13.2	178	92.5

NOTE: No *S. indica* appeared in control air samples taken before each test. Organisms passing through the barrier were collected with sieve samplers for 5 minutes at 1 cfm. Liquid impingers were used to determine the number of organisms per cubic foot of air at the point of nebulization. The collection efficiency of these samplers is estimated at 95 per cent for the liquid impinger and 45 to 70 per cent for the sieve sampler.

Figure 41. Vinyl Plastic Curtain. (FD Neg C-3700)

The use of germicidal radiation to prevent cross-infection of animals has been reported by Lurie (195) who irradiated an area between wire cages housing rabbits with tuberculosis and cages housing normal rabbits, thus preventing the 71 per cent cross-infection rate which occurred without the use of UV. UV radiations have also been used by Henle et al (138) to protect laboratory animals from air-borne infection. We have devised and evaluated a method of attaching UV lamps and fixtures to animal cage racks which provides a radiation barrier across the open top of solid-sided animal cages and thereby prevents the escape of a portion of the air-borne organisms from within the cage.

1. Materials and Methods

UV cage rack: The UV cage rack, shown in Figure 42, is 5 feet high, 4 feet wide, and 22 inches deep with solid metal shelves. Two 15-watt, 18-inch hot cathode UV lamps with fixtures are needed for each shelf. Each fixture is equipped with a reflector of Alzak (Aluminum Company of America) aluminum to direct the radiation in a band across the top of the cages. The fixtures are attached to the upright angle-iron corners at the side of the cage rack and are adjustable to any height above the shelf.

Only cages of equal height can be used on any one shelf. Cages must have solid sides to protect the animals from radiation. The position of the UV fixtures is adjusted so that the bottom edge of the lamps is level with the top edge of the cages. If large, open-top cages are used (e.g., a cage large enough to occupy one entire shelf) it may be necessary to place the bottom edge of the lamps slightly below the level of the top of the cage to prevent excess radiation from entering the cage. This is not necessary when screen wire or hardware cloth cage tops are used because these materials reduce the entrance of radiant energy. The underside of each shelf should be painted with a low-reflecting, nongloss paint to reduce reflectance of radiation into the cages.

Test bacteria used were Serratia indica and spores of Bacillus subtilis var. niger. For the former, 24-hour broth cultures grown at 27°C were used. Spore preparations were obtained by heat shocking, for 10 minutes at 80°C, the growth harvested from heavily inoculated tryptose agar plates or broth incubated for 5 days at 37°C.

Recovery of test organisms from the air was accomplished by means of sieve air samplers operated at the rate of one cubic foot per minute, and liquid impingers operated at 0.5 cubic foot per minute. The plating medium used in all tests was corn steep agar, adjusted to pH 7.0 to 7.5 before sterilization. Aerosols of the test organisms were produced with a DeVilbiss No. 40 nebulizer or a Chicago-type atomizer (260). Stainless steel animal cages, 9 inches x 10 inches x 18 inches, without tops or with one-fourth-inch wire-mesh tops were used on the cage racks.

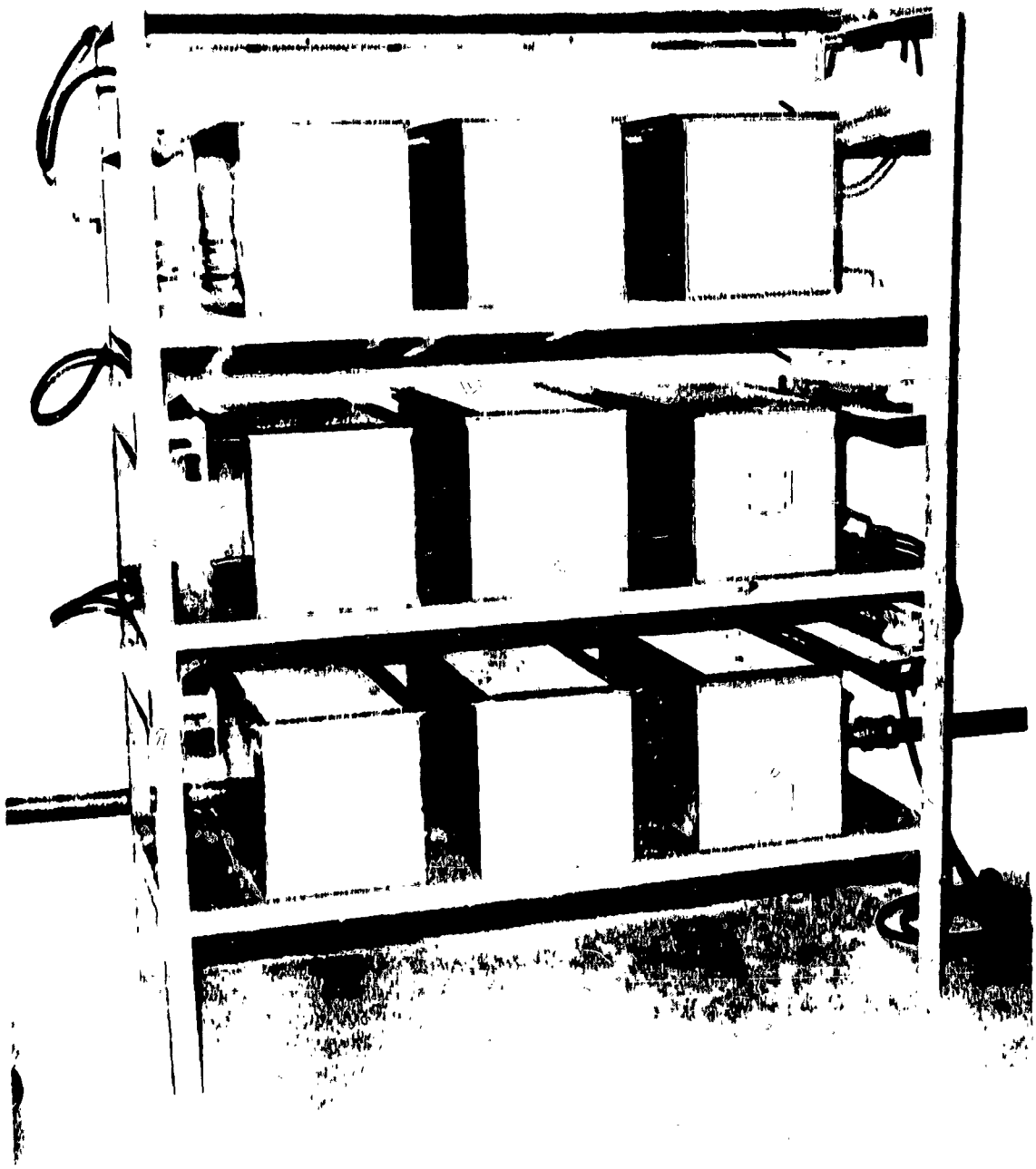


Figure 42. PV Cargo Rack (Pre Mog Bed)[30]

Operating efficiencies of all UV lamps used in the tests were checked by means of a SM-600 ultraviolet meter. Intensity readings at various positions on the rack were taken with a Luckiesh-Taylor germicidal meter or with SM-200 ultraviolet meter and expressed in terms of microwatts per square centimeter. To standardize the UV intensity each lamp was "seasoned" by burning for 100 hours before use. Before each experiment careful measurements were made of the radiation present above the cages. Typical intensities in microwatts per square centimeter are shown in Figure 43. The energy flux, or intensity recorded by the meter from three directions, was at least 250 microwatts per square centimeter. This figure has been used as a "yardstick" to assure that UV cage racks are operating correctly.

Preliminary experiments with artificially produced aerosols of S. indica were conducted in the absence of cage liter, animal hair, and debris. Empty animal cages, without tops, were placed on UV cage racks and aerosols were produced in such a manner that when air samples were taken with the UV lamps on and then off a comparison could be made of the ratio of bacteria inactivated.

Further experiments utilized secondary aerosols produced by organisms naturally liberated from the hair of guinea pigs exposed to primary aerosols of test bacteria. Four guinea pigs were exposed bodily to aerosols of S. indica or B. subtilis spores for five minutes in a sealed cabinet. The bacteria per cubic foot of air, as determined by liquid impinger samplers, averaged 3×10^7 per cubic foot for S. indica and 5×10^7 per cubic foot for B. subtilis spores. Immediately after exposure, each animal was transferred in a sterile closed cage to the UV cage rack. Two cages, each with a single guinea pig, were placed on the top shelf and two on the second shelf. Mesh-wire tops were then substituted for the closed tops and sieve air samplers were operated at the 9 positions shown in Figure 44. When the effect of UV radiation was to be tested, the lamps were turned on 30 minutes before the cages were placed on the racks. Five cubic feet of air were sampled at each sampling station at the following intervals: (a) just before placing cages on the rack, in the absence of UV (this was a control for the presence of test organisms in the room); (b) immediately after placing cages on the rack; (c) 30 minutes after exposure of the guinea pigs to the aerosol; (d) 60 minutes after exposure; and (e) 240 minutes after exposure. Each test and control was repeated at least seven times for each test organism.

2. Results

a. Experiment I - Artificially Produced Aerosols

An empty open-top cage was placed in the center of a shelf on a UV cage rack. S. indica culture was nebulized in the bottom of the cage and four sieve air samplers were operated one foot from the cage during alternate on and off periods of the UV lamps. The results obtained in five replicate experiments are given in Table XLV. The UV radiations prevented the escape of 97 to 100 per cent of the organisms which, in the absence of UV, were recovered by the four air samplers.

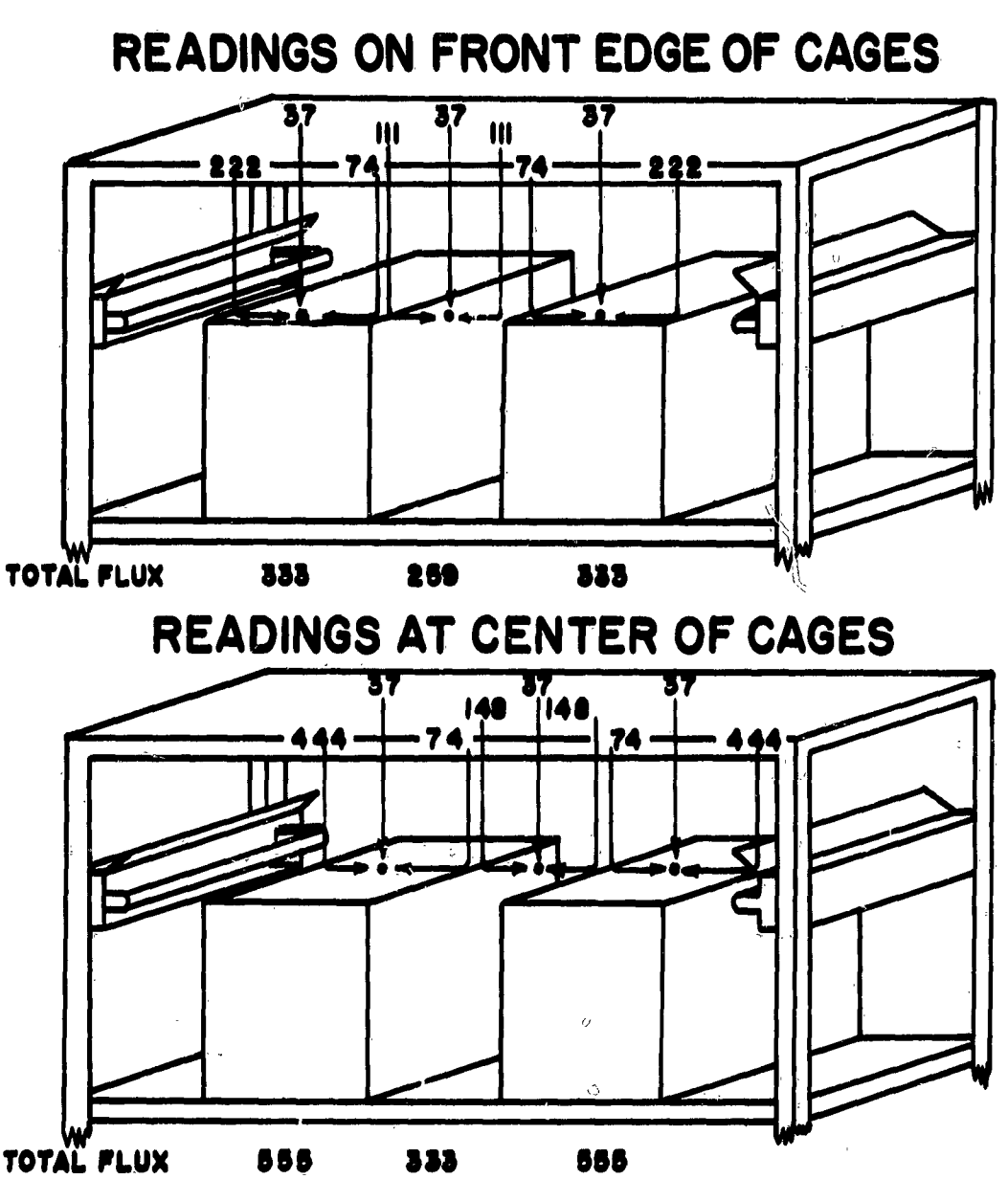


Figure 43. Typical UV Intensities Obtained on an Animal Rack Shelf Equipped with Two Cages and Two UV Fixtures. Arrows indicate the direction of the radiation. Readings are given in microwatts per sq cm.

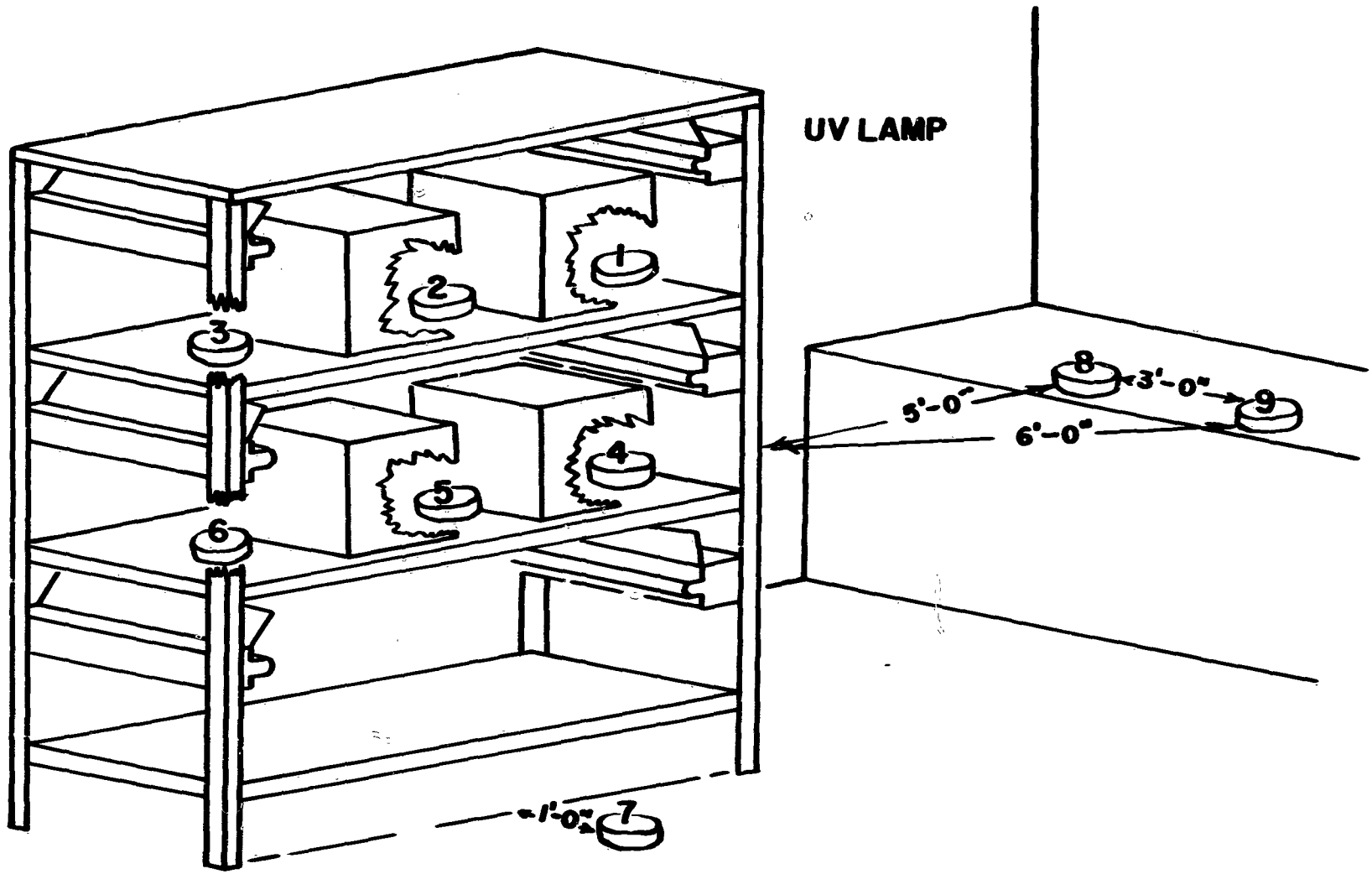


Figure 44. UV Animal Cage Rack. Positions of Sieve-Type Air Samplers.

TABLE XLV. PASSAGE OF ARTIFICIALLY PRODUCED AEROSOLS OF S. INDICA FROM A CAGE ON AN UV RACK TO THE ROOM[a]

	TOTAL COLONIES COLLECTED IN 5 MINUTES[b]		PER CENT REDUCTION BY UV
	UV Off	UV On	
Test 1	1200	0	100
Test 2	1267	34	97

a. Control air samples taken immediately prior to nebulization were negative for S. indica.
b. Average of five experiments.

The second test was concerned with cross-contamination between cages. S. indica was nebulized in the bottom of the center cage of three open cages on the center shelf of a UV cage rack while air samples were being taken by means of sieve samplers in the bottom of the other cages. Nebulization was continuous throughout the test. The results are shown in Table XLVI. Cross-contamination between cages did not occur when the lamps were on; no colonies grew on the sample plates in three duplicate tests. Air samples taken when the lamps were off always showed S. indica colonies too numerous to count.

TABLE XLVI. PASSAGE OF ARTIFICIALLY PRODUCED AEROSOLS OF S. INDICA FROM CAGE TO CAGE ON AN UV RACK[a]

	COLONIES COLLECTED IN CAGES		PER CENT REDUCTION BY UV
	UV Off	UV On	
Test 1	TNTC[b]	0	100
Test 2	TNTC	0	100
Test 3	TNTC	0	100

a. Control air samples taken immediately prior to nebulization were negative for S. indica.
b. Too numerous to count.

b. Experiment II - Secondary Aerosols

Tables XLVII and XLVIII and Figure 45 summarize results obtained with secondary aerosols. Guinea pigs whose hair was contaminated with S. indica or with B. subtilis spores released a considerable number of organisms into the room. In the absence of UV an average of 479 colonies of S. indica and 1,824 colonies of B. subtilis per 5 cubic feet of air was obtained by the 9 samplers immediately after placing the exposed animals on the cage rack. Air-borne organisms were not confined to the immediate area of the cage rack because recoveries were obtained by the samplers located five and six feet from the rack. Control air samples taken immediately prior to the beginning of each test were negative for S. indica and B. subtilis.

TABLE XLVII. EFFECT OF UV RADIATION ON AIR-BORNE S. INDICA RELEASED FROM THE HAIR OF GUINEA PIGS

SAMPLING TIME AFTER START OF TEST	NUMBER OF COLONIES COLLECTED[a]		PER CENT REDUCTION BY UV[b]
	UV Off	UV On	
0-5 minutes	479	13.0	97.3
30-35 minutes	150	1.4	99.1
60-65 minutes	104	0.8	99.2
240-245 minutes	84	0.3	99.6

a. Average of 8 experiments.
b. Obtained by comparing the recoveries with the UV on and off at each sampling time.

TABLE XLVIII. EFFECT OF UV RADIATION ON AIR-BORNE B. SUBTILIS SPORES RELEASED FROM THE HAIR OF GUINEA PIGS

SAMPLING TIME AFTER START OF TEST	NUMBER OF COLONIES COLLECTED[a]		PER CENT REDUCTION BY UV[b]
	UV Off	UV On	
0-5 minutes	1824	1663	8.8
30-35 minutes	1442	864	40.1
60-65 minutes	1168	626	46.4
240-245 minutes	674	350	48.1
		(UV turned off)	
250-255 minutes	---	614	---

a. Average of 7 experiments.
b. Obtained by comparing the recoveries with the UV on and off at each sampling time.

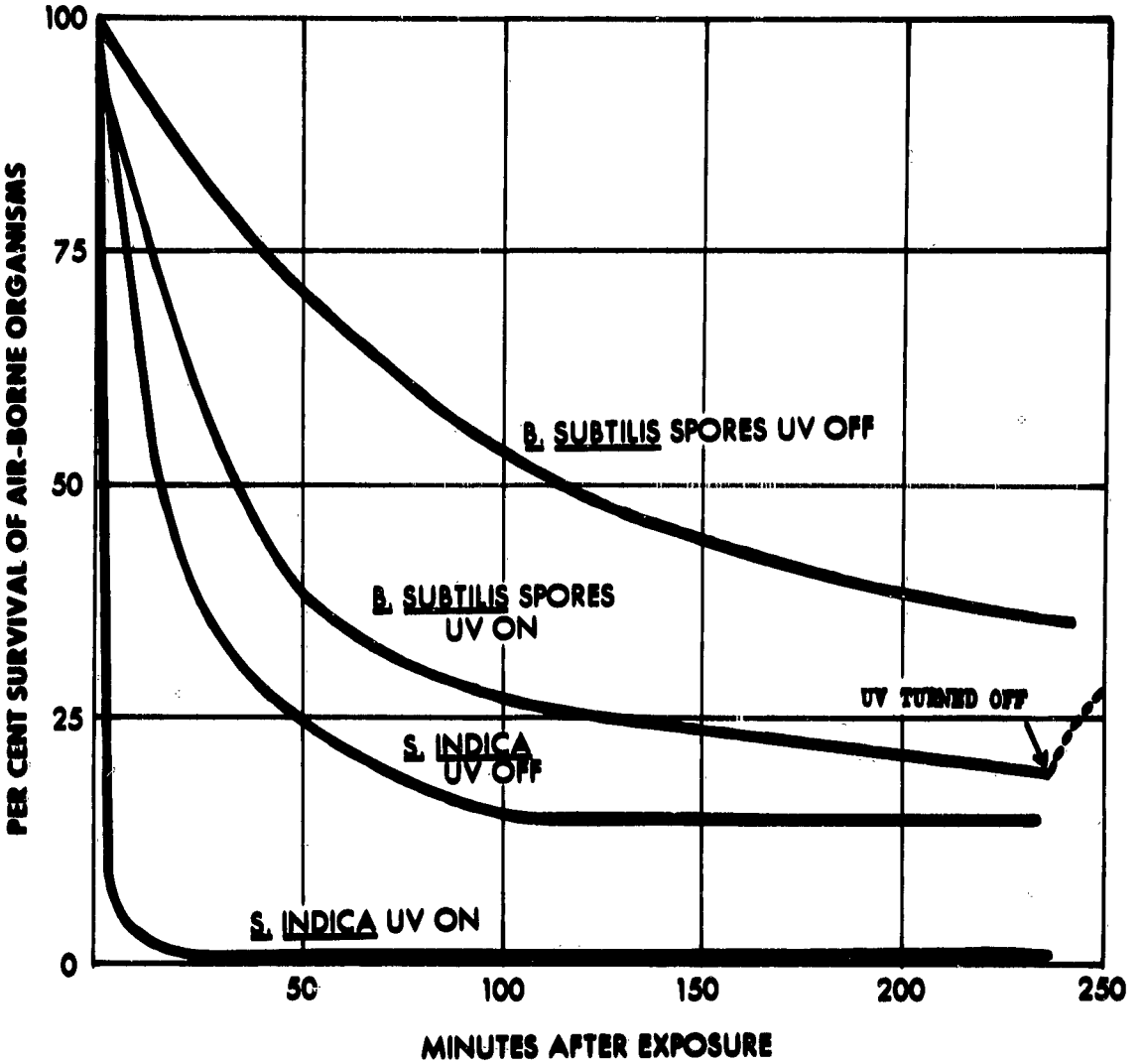


Figure 45. Recovery of Air-Borne S. indica Cells and B. subtilis Spores Released from Hair of Four Aerosol-Exposed Guinea Pigs Housed in Solid-Sided, Screened-Top Cages Placed on an UV Cage Rack.

In the absence of the UV barrier, air-borne spores were still present in large numbers and the vegetative organisms, to a lesser degree, 240 minutes after exposure of the animals to the aerosol. The striking effect of the UV barrier on the vegetative organisms (Table XLVII) is shown by almost complete elimination of recoverable bacteria. By comparison, although there was a considerable reduction of air-borne spores, a large amount still survived the radiation treatment (Table XLVIII). The barrier effect, however, is clearly shown by the sharp rise in recoverable spores obtained five minutes after the UV was turned off (Figure 45).

The reductions in recovery of air-borne S. indica and B. subtilis spores from aerosol-exposed guinea pigs brought about by the use of the UV lamps on the cage rack are summarized in Table XLIX. With S. indica, reductions ranged from 97 to 99 per cent. With B. subtilis spores, the reductions ranged from 9 to 48 per cent.

TABLE XLIX. EFFECTIVENESS OF THE UV CAGE RACK IN REDUCING THE NUMBER OF S. INDICA CELLS AND B. SUBTILIS SPORES RELEASED FROM WITHIN THE CAGE FROM HAIR OF GUINEA PIGS

INTERVAL BETWEEN AEROSOL EXPOSURE OF ANIMAL AND AIR SAMPLING, minutes	PER CENT REDUCTION	
	S. indica	B. subtilis Spores
0	97	9
30	99	40
60	99	46
240	99	48
Average Reduction	98.5	35.6

3. Discussion

These studies demonstrate the usefulness of germicidal radiation as a barrier over animal cages to reduce the entrance and exit of air-borne organisms. Although there was only a slight difference in the amounts of vegetative organisms and spores in the aerosols to which the guinea pigs were exposed, the secondary aerosols produced with spores were considerably greater. This probably can be accounted for by the greater survival ability of the spore. The fact that the UV barrier had a reduced effect on the spores is not surprising because Duggar and Hollaender (71) observed B. subtilis spores to be several times as resistant to UV as the vegetative cells of the same strain. This was confirmed by Herdik (140) with

B. megaterium and by Rentschler et al (247) with B. subtilis, and by experiments by the authors described on page 168. The incident energy at 2537A necessary to inhibit colony formation in 90 per cent of the organisms for B. subtilis spores is sixfold over the energy necessary to inhibit S. marcescens (151).

The value of the UV cage rack has been demonstrated in our laboratories over a period of approximately eight years. In animal rooms containing 10 to 20 UV cage racks, the radiation level in the room is sufficient to eliminate almost all air-borne and many surface organisms. No trouble with animal epidemics has been observed and cross-infection between cages has not been noted. Some stray radiation enters the cages and although these radiations have been found to inactivate S. indica on the surface of agar plates, no deleterious effects of UV radiation have been noted on the eyes or exposed skin of animals.

Animal workers must wear skin and eye protection when working in rooms with UV cage racks. In our laboratories ventilated personnel hoods (Snyder Manufacturing Company, New Philadelphia, Ohio) are worn for skin and eye protection (Figure 46).

E. ULTRAVIOLET IN INCUBATORS AND REFRIGERATORS

1. Effect of UV Radiation on Stored Cultures

In considering the applicability of germicidal UV radiation for use in incubators, refrigerators, cabinets, and other enclosed spaces several phenomena must be investigated and any resulting limitations applied in practical installations. The pertinent points for consideration are:

(a) Will cultures being used, stored, or incubated under UV radiation be adversely affected because of penetration of the germicidal radiation, through the walls of the containing vessel?

(b) What detrimental effects may be caused by the ozone produced by UV lamps?

a. Preliminary Experiments

A series of preliminary experiments were conducted in refrigerators and incubators. One shelf in a walk-in type refrigerator room was equipped with two overhead 30-watt, hot cathode lamps located 20 inches above the shelf. The purpose of the tests was to detect any detrimental effects to glass enclosed cultures when stored under the radiations at 5°C or when incubated under the radiations at 37° or 30°C.

Figure 46. Ventilated Personnel Hood. (FD Neg C-3701)

A 5.5-cubic foot incubator was equipped with a 15-watt hot cathode UV lamp in its ceiling. A similar incubator without the UV lamp was used for the control tests. Agar and broth cultures of S. indica and Brucella abortus strain 19 were used as test organisms. Incubation was carried out on two shelves. The bottom shelf was located 17 inches from the radiation source and received an average UV intensity of 150 microwatts per square centimeter. The top shelf was eight inches below the UV lamp and received an average of 250 microwatts per square centimeter.

Test organisms were inoculated onto the surface of agar plates or into flasks of broth; duplicate samples were incubated in the two incubators and the total numbers of organisms or colonies obtained were compared. Agar plates were first incubated agar side down. It was found that cultures incubated under these intensities of UV radiation showed lower numbers of colonies or of total cells than the control cultures. The decrease ranged from 10 to 90 per cent, the higher figure being obtained with cultures on the top shelf. Because ordinary laboratory glassware is opaque to 2537A radiation, it was assumed that the reduction in cell yield was due to penetration of the glass by the small percentage of longer waves in the erythemal range. The germicidal effectiveness of these radiations is less (Table XII) than that of UV radiation in the 2537A range, but the long exposure time provides a sufficient dose to effect a noticeable killing. A covered set of controls, which was included in the incubator with the UV lamp with each experiment, showed that very little or none of the lethal action was due to the presence of ozone. When the UV intensities in the incubator were reduced to 100 microwatts per square centimeter or less, very little inhibition of the two test strains was noted.

The average intensity of UV radiation falling upon the test shelf in the walk-in refrigerator room was 280 microwatts per square centimeter. When broth cultures of S. indica and B. abortus strain 19 were stored at 5°C under the lamps for periods as long as 42 days, no significant reduction in the number of viable cells was found. The control cultures were stored at the same temperature but not exposed to the radiation.

The above results are conflicting because an intensity of 250 microwatts per square centimeter for 42 days in the refrigerator showed no deleterious effects on stored test organisms, whereas in the incubator tests, 150 microwatts per square centimeter for two or three days yielded counts 10 to 90 per cent below that of the control cultures. It has been shown, however, that UV has a more deleterious effect on young growing cultures than on old resting cells.

b. Quantitative Experiments

Further tests were made to determine quantitatively the inhibitory effects of various amounts of germicidal energy on Petri dish cultures.

The tests were conducted in an air-conditioned room containing a recirculating air blower and sufficient natural ventilation to remove all traces of ozone. S. indica was used as the test organism. Inoculated agar plates (closed) were exposed and incubated on open shelves in the room where the temperature (25°C) permitted good growth of the test organism in 24 hours. The intensity of the radiation falling on the dishes was varied by changing the distances from the UV lamp to the shelf below. The Petri dishes were incubated agar side up and agar side down.

An inoculum sufficient to give 30 to 300 colonies per Petri plate was spread evenly over the surface of a group of agar plates. Each plate received the same inoculum. One-half of the inoculated plates were controls (shielded from the UV radiation). The remaining plates were incubated adjacent to the controls and exposed to UV radiation from above. The plates were placed so that each received the same amount of radiation. The Petri dishes were allowed to incubate under these conditions for 18 to 24 hours. The colonies were then counted and compared with the total number of colonies appearing on the exposed and the nonexposed agar plates. The test was repeated a number of times to insure consistent and reproducible results. Four radiation intensity levels were used.

c. Plates Exposed Agar Side Up

It is a general practice to incubate agar plates with the agar side up (inoculated surface toward the bottom) to prevent excessive moisture condensation on the agar surfaces. If plates incubating in this manner are irradiated from above, light waves must penetrate both the Pyrex glass and the agar before reaching the growing bacterial cell.

Fifteen to twenty milliliters of melted corn steep agar were used in each Petri dish. The solidified agar was dark brown in color and translucent to white light. A total of 26,662 S. indica agar colonies were counted. Of these, 13,325 colonies were counted on control plates not exposed to UV radiation but exposed in the same room, and consequently to any ozone present. The remaining 13,337 colonies were counted on an equal number of plates exposed agar side up during incubation to four different UV intensities. The results of these tests and the intensities used are shown in Table L.

Intensities of UV as high as 400 microwatts per square centimeter did not result in detectable inhibition. There was no noticeable difference in the size or pigment in the S. indica colonies counted. Apparently, 2537A radiation at this intensity level has no effect when directed toward the agar side of an incubating Petri dish.

TABLE L. GROWTH OF S. INDICA COLONIES ON AGAR SURFACES WHEN THE PETRI DISHES WERE EXPOSED AGAR SIDE UP TO UV RADIATION

UV INTENSITY, microwatts per sq cm	NUMBER S. INDICA COLONIES COUNTED		
	Control Plates	Exposed Plates	Total
100	3104	3040	6144
300	3504	3652	7156
350	3285	3353	6638
400	3431	3292	6723
Over-all totals	13325	13337	26662

d. Plates Exposed Agar Side Down

When incubated agar plates are placed agar side down and irradiated from above, the agar colonies are inhibited according to the intensity of the radiation falling upon the glass surface. These tests were performed in exactly the same manner as the previous tests except that all plates were placed agar side down.

A total of 48,584 colonies were counted during the tests. Of these, 28,000 were counted on control plates not exposed to UV radiation. The remaining 20,684 colonies appeared on an equal number of plates that had been incubated agar side down but exposed to four UV intensities. As shown in Table LI, a reduction of almost 70 per cent was obtained on the plates exposed to 400 microwatts per square centimeter 2537A radiation while at 100 microwatts per square centimeter the reduction was six per cent. In addition, colonies appearing on the agar surfaces tended to be smaller than the control colonies and showed a pink instead of a red pigment. This effect was more marked as higher intensities were used. Thus, if inoculated agar plates are incubated agar side down and irradiated with 2537A radiation at intensities of 100 microwatts per square centimeter or more, inhibition of bacterial growth will occur.

At first glance the results of these bacteriological tests seem to conflict with the data which show the inability of Pyrex and other types of glass to transmit 2537A UV. The bacteriological tests were conducted in such a way that inhibition by ozone was impossible. If Pyrex Petri dishes do not transmit wave lengths of 2537A, how may the inhibition of the exposed test cultures be explained? The most logical explanation is that the reduction in count on the exposed Petri plates was a result of penetration of wave lengths other than 2537A. The germicidal UV lamp produces 95 per cent of its radiation in the 2537A band. The remaining five per cent consists of longer and shorter radiation. Table X shows that the

lamps emit 0.22 per cent of the total energy in the 3022A band, 1.90 per cent in the 3130A band, and 2.0 per cent in the 3650A band. A sheet of Pyrex glass three millimeters in thickness (Table XII) will transmit 21, 47, and 89 per cent respectively of the light of these wave lengths. Also, the estimated relative germicidal effectiveness of 3650A is 0.25 per cent. With the long exposures possible during incubation, it is likely that the detrimental effects demonstrated in these tests can be attributed to the action of radiation longer than 2537A.

TABLE LI. GROWTH OF S. INDICA COLONIES ON AGAR SURFACES WHEN THE PETRI DISHES WERE EXPOSED AGAR SIDE DOWN TO UV RADIATION

UV INTENSITY, microwatts per sq cm	NUMBER S. INDICA COLONIES COUNTED			PER CENT REDUCTION BY UV RADIATIONS
	Control Plates	Exposed Plates	Total	
100	14193	13291	27484	6.35
300	7170	4930	12000	31.24
350	3388	1329	4717	60.77
400	3249	1034	4283	68.17
Over-all totals	28000	20684	48684	26.13

That such a supposition is possible may be emphasized by considering a theoretical example. If a certain culture is inactivated by direct exposure to 2537A UV at an intensity of 100 microwatts per square centimeter for one minute, then 1.90 per cent of this radiation will be of wave length 3130A. Suppose that wave length 3130A is one per cent as effective as 2537A. If the culture is covered with a sheet of Pyrex glass, none of the 2537A radiations will penetrate, and 47 per cent of the 3130A wave lengths will be transmitted. Therefore, the equivalent germicidal UV radiation reaching the culture would be 0.00893 microwatts per square centimeter and, in 11,198 minutes or 186.6 hours, the covered culture would receive a dosage of 3130A radiation equal in germicidal activity to the original direct exposure of 100 microwatts of 2537A per square centimeter for one minute. Thus, although Pyrex glass will not transmit light of wave length 2537A, penetration of the longer UV radiation for long periods of time may show definite germicidal action.

e. Conclusions

The regular 15- or 17-watt UV lamps should not be used in non-walk-in type incubators and refrigerators. If a smaller size of lamp is used the intensity should be below 100 microwatts per square centimeter.

It is desirable to use UV lamps in walk-in type incubators, refrigerators and hoods, but care must be taken that the lamps are installed in such a manner that the maximum intensities reaching glass enclosed cultures is less than 100 microwatts per square centimeter. Bacterial cultures should be kept at distances from UV lamps greater than the following:

(a) 15-inch, hot cathode lamp: cultures to be kept at a distance of 18 inches or more;

(b) 36-inch, hot cathode lamp: cultures to be kept at a distance of 36 inches or more; (Hot cathode lamps are not designed to be used in refrigerators.)

(c) 34-inch, cold cathode lamp: cultures to be kept at a distance of 24 inches.

2. Effects of UV Radiation in a Walk-In Incubator

Conditions in walk-in incubators are generally favorable for the survival or growth of contaminating microorganisms. Since incubators usually are not ventilated, the microbial population may be quite high. When infectious cultures are incubated, escape of pathogens from broken flasks or from flasks with missing stoppers may constitute a hazard to persons entering the incubator. Breakage or spillage on a shaking machine or from a culture aeration apparatus may be especially dangerous.

Evaluation studies were made of the effectiveness of UV radiation in reducing surface and air-borne microbial flora in a 9 x 8-foot walk-in incubator room with an 8-foot ceiling. Triplicate samples of air and surfaces in the room (30°C), were taken for six days under three separate conditions and examined for common bacteria and fungi. The conditions were:

(a) Control - no UV.

(b) Indirect UV - one 17-watt cold cathode UV lamp mounted eight inches below the ceiling in the center of the room and shielded to irradiate upwards.

(c) Indirect and direct UV - condition (b) plus one 17-watt lamp mounted 12 inches below the ceiling and irradiating downward.

Indirect UV radiation of low intensity (due mostly to reflectance) was present on the upper shelves in the room, but no radiation reached the floor. When both lamps were burning, 17 to 82 microwatts per square centimeter of radiant energy were present on the shelves, and the exposed floor area received approximately 13 microwatts per square centimeter (Figure 47).

During the six-day test for each condition, normal use of the incubator was continued. The bacteria and fungi recoverable from the air by sieve samplers and from the walls by moistened sterile swabs during the test periods were reduced by 83 to 100 per cent as compared to the controls (Table LII). Indirect UV radiation reduced the number of microorganisms on the floor only slightly. Direct radiation caused an 86.5 per cent reduction in floor bacteria on exposed surfaces, but the reduction in numbers of fungi was not determined because of overgrowth by bacteria in the control samples.

TABLE LII. REDUCTION OF ORGANISMS BY CONTINUOUS UV RADIATION IN AN INCUBATOR ROOM (30°C)

CONDITION TESTED	PER CENT REDUCTION BY ULTRAVIOLET[a]			
	Indirect Ultraviolet		Direct and Indirect Ultraviolet	
	Bacteria	Fungi	Bacteria	Fungi
Air-borne organisms	83.4	84.7	91.8	84.7
Organisms on the floor	---[b]	---[b]	86.5	---[c]
Organisms on the walls	99.4	92.0	100	100

a. Averaged from samples in triplicate taken on each of 6 days.
b. Very little reduction.
c. Reduction not determined.

Obviously, when UV is used, the microbial population is reduced and then remains rather constant. Equilibrium conditions were maintained although normal use of the incubator continued. When indirect UV was used, all air samples were taken close to the floor where no radiation was present. Air circulation was therefore responsible for lower air counts in all parts of the incubator. Of course, no decontamination occurred on surfaces not exposed to radiation.

The reduction of fungi was about the same as for bacteria, in spite of the fact that molds are considered to be 100 to 1,000 times as resistant as bacteria (174). This parallelism suggests that the exposure times were sufficient to kill even the hardiest microorganisms, and, in reality, the limiting factor for destruction was the ability or inability of the radiation to reach the cells.

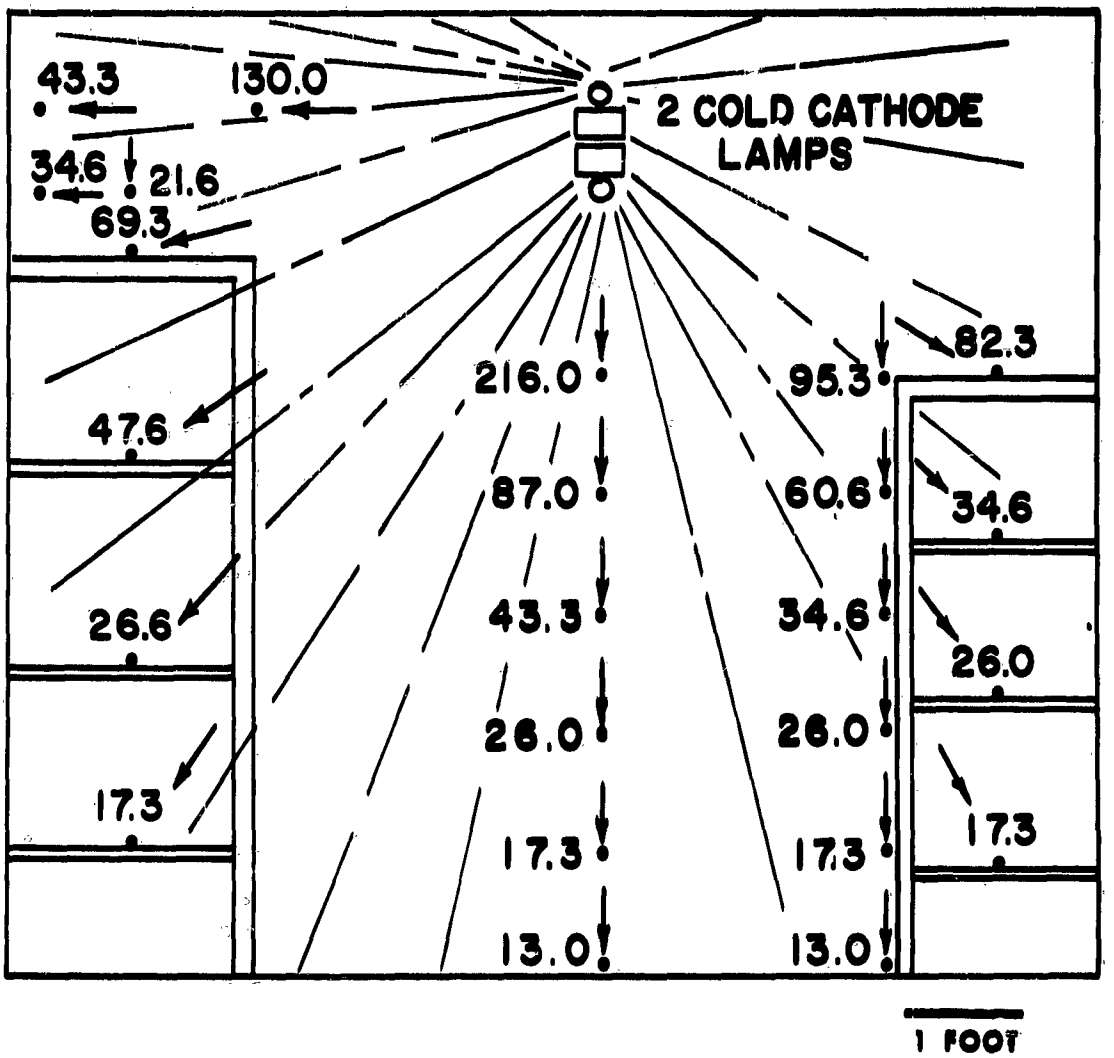


Figure 47. UV Intensities in Microwatts Per Sq Cm in an Incubator Room. Two Cold Cathode Lamps. Arrows indicate the direction of the radiations. Sketch represents a vertical section through the center of the room.

Besides constantly functioning to eliminate ordinary bacteria and molds in walk-in incubators, UV lamps should be capable of reducing or eliminating aerosols of infectious agents that might be suddenly produced in a room by an accident. Tests were carried out to evaluate the effectiveness of the UV lamps in the test room when large numbers of test organisms were suddenly released. Broth cultures of S. indica were nebulized in the incubator room under the same conditions of UV exposure as in the previous tests. Sieve samplers were used to recover the test organisms. Four milliliters of a S. indica culture (2×10^7 cells per ml) were nebulized and air samples taken at various time intervals, beginning at the start of nebulization.

The results are shown in Table LIII. During the control run (UV lamps off), test organisms were present in the air one hour after nebulizing, but not after two. The use of the indirect UV lamp eliminated all recoverable air-borne S. indica in less than ten minutes. Using both the direct and indirect UV radiation, a rapid kill of the organisms was obtained, as evidenced by the fact that no viable cells could be recovered during the first sampling period (zero minutes). The nebulizer was located in the center of the room, about three feet from the floor.

UV radiation is not recommended for incubators if it is critically important to preserve the genetic or nutritional characteristics of the microorganisms in use. In other situations, however, the use of germicidal radiation can provide good protection in walk-in type refrigerators and incubators to control hazards created by the escape of infectious microorganisms.

TABLE LIII. RECOVERY OF S. INDICA IN A WALK-IN INCUBATOR DURING AND AFTER NEBULIZATION AND THE EFFECT OF UV RADIATION ON RECOVERY

TIME, minutes	NUMBER OF AIR-BORNE S. INDICA CELLS RECOVERED PER CU FT		
	Condition		
	UV Off	Indirect UV On	Direct and Indirect UV On
0	60	60	0
10		0	0
20		0	0
60	44.8	0	0
120	0	0	0

F. THE UV DECONTAMINATION CHAMBER

The UV decontamination chamber is a two door, rectangular shaped, stainless steel box, designed to be installed in the wall or door separating contaminated areas from clean areas (Figures 48 and 49). The purpose of the chamber is to provide a mechanism by which small articles can be transferred from clean to potentially contaminated areas and by which sheets of paper and similar small articles may be decontaminated and passed from contaminated to clean areas. The doors, which comprise the two ends of the box, have small sight glasses. The over-all dimensions are: height, 38 inches; width, 22 inches; and depth, 16 inches. Two 36-inch, 30-watt hot cathode UV lamps are mounted vertically on the inside of each door. An activating switch with blue indicator light, is located on the outside of each door enabling operators to turn the lamps off or on from either side. The small window can be used to see if all lamps are lit.

The presence of the four UV lamps in the cabinet causes a considerable rise in temperature within the chamber. When the temperature of the outside air is approximately 25°C, a temperature of 44°C is reached within the chamber.

High intensities of UV radiation are present throughout the chamber; from 500 to 1500 microwatts per square centimeter. The vertically mounted lamps extend almost the entire length of the chamber and consequently there is very little shadowing when papers are placed on the horizontal shelves. Except for the small area where the sheets of paper rest upon the shelf, the entire sheet is irradiated.

1. Effect of UV Radiation on Plastic Recording Discs

Plastic recording discs are sometimes used in contaminated areas and after use, they are taken to the clean area. Voice transcriptions were made on plastic discs which were then placed in the UV chamber for a period of 48 hours. The exposure caused the discs to change from a light grey to a dark green. No brittleness or stickiness was observed and they were otherwise in perfect condition. On the playback machine, the clearness and volume of the voice was the same as on an unexposed transcription.

2. Decontamination of Plastic Recording Discs

Plastic recording discs were artificially contaminated with several million cells of S. indica, exposed in the chamber for a short period of time and subjected to tests to recover viable cells. No S. indica was recovered after ten minutes exposure time in the UV chamber.

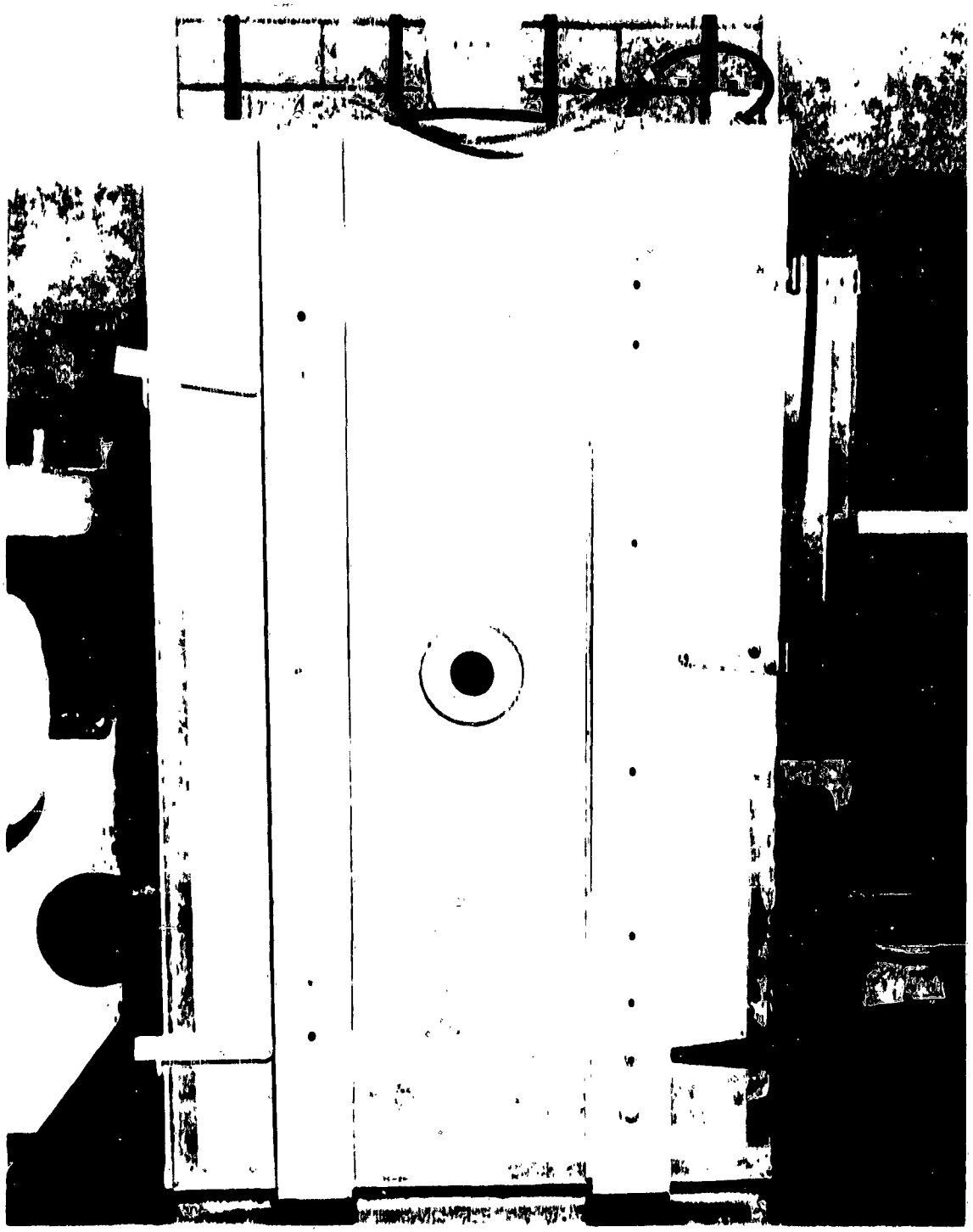


Figure 48. UV Paper Decontamination Chamber, Closed. (FD Neg B-6217)

Figure 49. UV Paper Decontamination Chamber, Opened. (FD Neg B-6216)

3. Effect of UV Radiation on Paper

Bond typing paper, notebook paper, and other types of paper available in the laboratory were exposed to radiation in the UV chambers for periods up to 24 hours. There was no effect other than a slight curling of the edges of the paper. Prolonged exposures (one week to one month) produced yellowing of paper and brittleness but were not considered important because the time necessary to decontaminate usually does not exceed ten minutes.

4. Decontamination of Paper

a. Experimental Tests

Tests were conducted using a smooth surface paper (bond typing paper) and a relatively rough paper (scratch pad paper). Test strips were sterilized and artificially contaminated with test organisms. After exposure in the UV chamber, the strips were placed in a small quantity of broth and an attempt made to recover viable cells.

In the first test S. indica was used as the test organism. The test strips were contaminated by simply wetting the surface with a broth culture of S. indica containing 1×10^9 cells per milliliter. Periods of 30 to 90 minutes of exposure were required to eliminate all viable cells.

Next, a broth suspension of B. subtilis var. niger spores was used to inoculate test strips. When heavily contaminated in this manner, irradiation in the chamber failed to inactivate all spores in reasonable periods of time. Strips of smooth paper were sterile in 72 hours.

Further tests involved paper strips that had been contaminated with atomized S. indica cells. A total of 60 smooth paper strips and 60 rough paper strips were exposed from 5 to 30 minutes in the UV chamber. For each type of paper, 10 strips each were exposed for 5, 10, 15, 20, 25, and 30 minutes. Unexposed controls were covered and placed in the chamber to insure that the observed effects were not due solely to the temperature.

All of the exposed smooth paper strips samples were sterile and all controls showed growth. All but one of the rough paper samples were sterile, and all controls were positive. The one positive rough paper strip was from a ten-minute exposure test and, since the five-minute exposures were sterile, this positive sample probably resulted from contamination occurring after the exposure. The five-minute exposure period was sufficient to sterilize both types of paper contaminated with S. indica.

In the UV chamber, the contact area of the paper to the shelf is kept to a minimum by using small, circular rods. During these tests, attempts were made to recover viable S. indica cells from the place of contact on contaminated test strips of paper. No viable cells could be recovered. It is logical, however, to assume that this would be a function of the degree of contamination of the paper.

b. Conclusions

(1) The UV chamber should not be used to treat papers contaminated with spore-forming microorganisms.

(2) It should not be used to treat papers known to be grossly contaminated, e.g., contaminated with liquid cultures.

(3) The chamber may be used to treat papers potentially contaminated with vegetative microorganisms.

(4) Papers to be treated should be separated individually so that all sides are exposed to the radiations.

(5) An exposure time of at least 10 minutes for each sheet of paper is recommended.

(6) UV lamps should always be turned off before opening the cabinet.

(7) The lamps should be cleaned frequently and replaced when they deteriorate.

G. UV PASS-THROUGH CHAMBER FOR SINGLE SHEETS OF PAPER

In infectious disease laboratories "contaminated" areas are sometimes physically separated from adjoining clean areas such as offices, libraries and conference rooms. In such instances it is desirable to disinfect or sterilize papers used for recording data in the laboratory before they are passed to the clean area. Although sterilization can be effected by autoclaving or by treatment with ethylene oxide gas (166), time or facilities may make these methods impractical. A pass-through chamber utilizing high intensity radiations has been developed for disinfecting single sheets of paper. This chamber eliminates objections to the chamber previously tested. The apparatus has been tested using strips of paper contaminated with four different species of organisms.

1. Materials and Methods

Exterior and interior views of the chamber are shown in Figures 50 and 51. The housing is fabricated from sheet aluminum and measures 24½ x 5 x 5 inches. When a single sheet of paper 15 inches or less in width is inserted in the slot, it is caught by two synchronously revolving rollers which push the paper at a controlled rate past four, 15-watt UV lamps. Each side of the sheet receives radiation from two lamps. The rollers are driven by a small ten revolutions per minute electric motor* which moves

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* Holtzer-Cabot Gear Head Motor, RWC 2505, Holtzer-Cabot Divisions, National Pneumatic Co., Inc., Boston, Massachusetts.

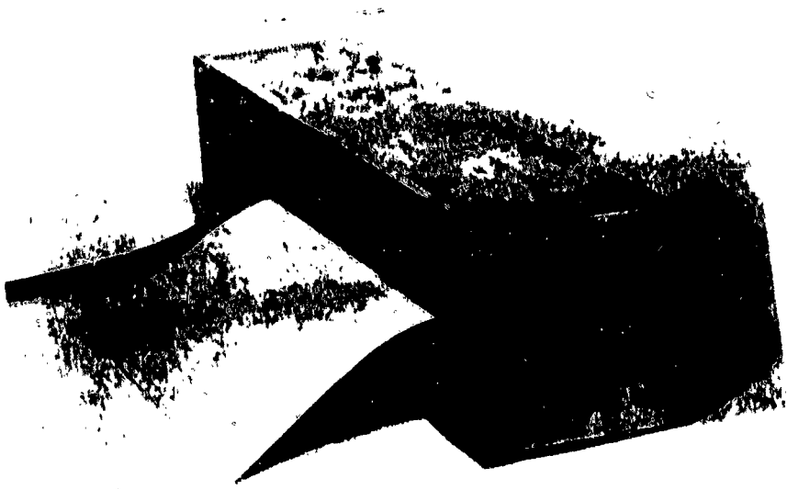


Figure 50. UV Paper Sterilizer (Single Sheet), Exterior View. (FD Neg B-8364)

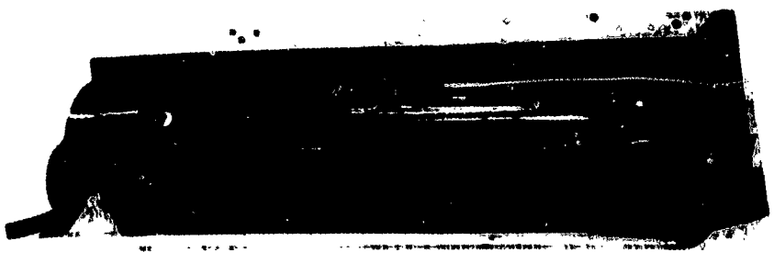


Figure 51. UV Paper Sterilizer (Single Sheet), Interior View. (FD Neg B-8363)

the paper at the rate of one inch every 3.25 seconds. The paper as it passes through the apparatus is subjected on each side to UV intensities varying from 8,000 to approximately 28,000 microwatts per square centimeter. The total UV radiation is about 7,500 microwatts-minutes per square centimeter or 4,500,000 ergs per square centimeter.

The chamber is designed for installation in a wall or doorway separating an infectious unit from a clean section. To facilitate maintenance, the front panel of the unit is hinged to permit removal of the inside structure (Figure 51). A switch located on the front of the chamber operates the motor and two of the UV lamps. The other two lamps burn continuously to prevent the passage of air-borne microorganisms. Laboratories in infectious disease units are often maintained under a reduced air pressure as compared to clean office areas to prevent cross-contamination. When the chamber is installed between areas of unequal air pressure, it is necessary to provide an air-tight catch box to receive the disinfected sheets of paper. A suitable catch box of clear Plexiglas is shown in Figure 52. The box has a bottom-opening door which utilizes a magnetic fastener. The design described herein represents the most effective of several developed and evaluated.

The effectiveness of the apparatus was tested by passing artificially contaminated strips of white bond paper, 8 inches x 1½ inches, through the machine and comparing the number of viable organisms remaining per square inch of paper with the number before UV treatment. Test organisms were Bacillus subtilis var. niger spores, Serratia marcescens, Escherichia coli B/r and T-3 coliphage. The strips were contaminated with spores by exposing them in a rectangular plastic cabinet (236) in which an aerosol of bacterial spores was generated. Liquid cultures of the other organisms were spread evenly over the paper strips. Unirradiated inoculated strips served as controls. Organisms were washed from the strips by shaking in 100 milliliters of sterile saline. Dilutions were made and aliquots were plated from the controls, but the entire 100 milliliters of wash fluid from the irradiated strips were cultured.

2. Results

The results are shown in Table LIV. The UV chamber was 100 per cent effective against S. marcescens and coliphage, and 99.97 per cent effective against bacterial spores.

To determine the possible effect of photoreactivation (158) on cells "killed" in the chamber, experiments were included in which UV resistant E. coli B/r cells on paper were treated with 2537A radiation and exposed to reactivating illumination in the visible or near-ultraviolet range. Without reactivating light, UV treatment in the apparatus reduced the cell concentration from 19,000 to 0.55 cells per square inch of paper (Table LIV). When UV treated cells were washed from the paper and exposed

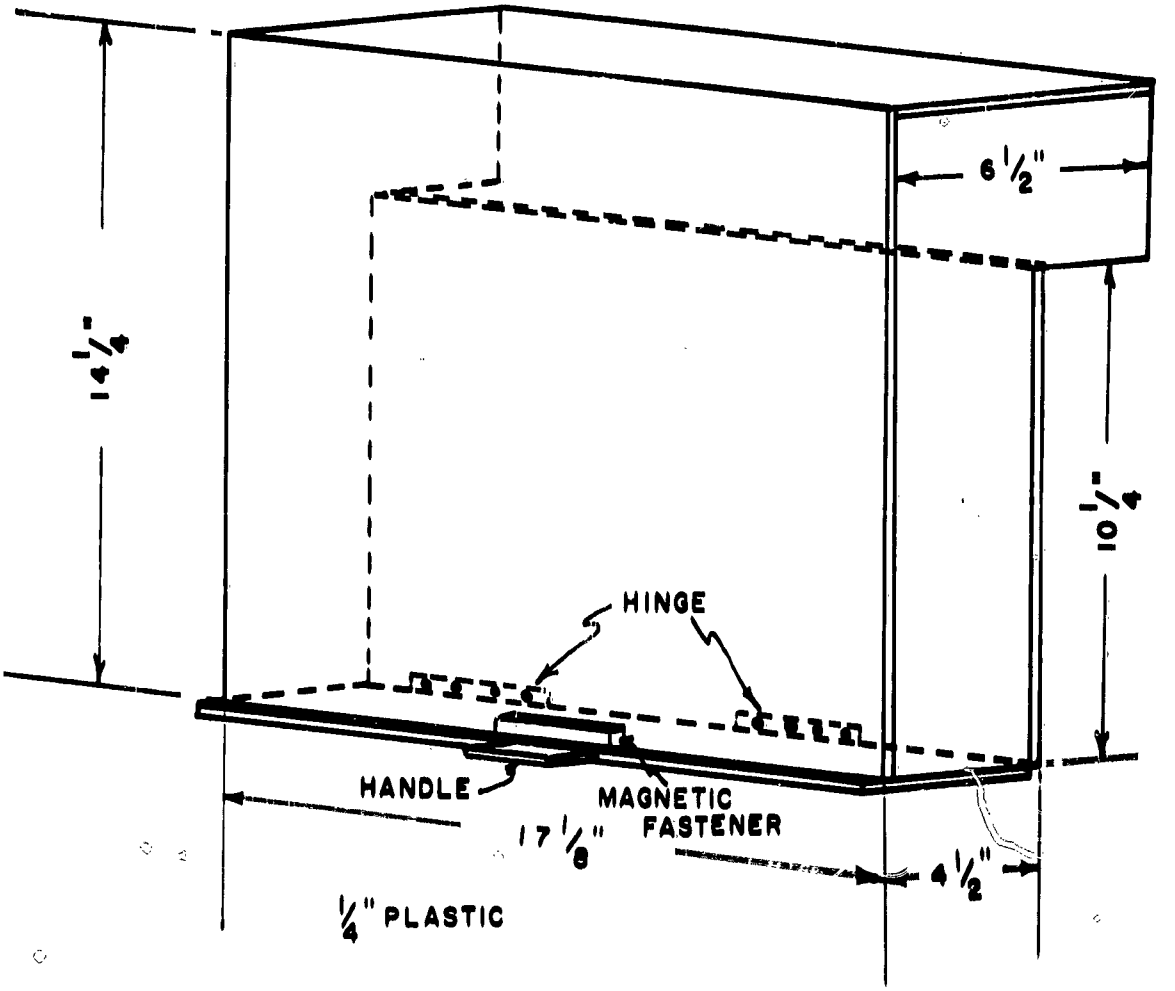


Figure 52. Receiving Box for UV Paper Steriliser.

for 50 minutes to illumination from daylight fluorescent lamps or fluorescent "black light" lamps (maximum spectral peak at 3660A), no significant increase in cell recovery was noted. The average kill as determined after exposure to UV plus reactivating light was 99.996 per cent and after UV alone 99.997 per cent. Using B/r cells exposed in Petri plates to radiation intensities lower than those obtained in the paper chamber, reactivation of a portion of the irradiated cells was demonstrated.

Although it is evident that the UV chamber will not provide sterilization of paper heavily contaminated with bacterial spores, it is believed suitable for normal operations in infectious disease laboratories. It is possible the UV chamber would prove useful in other types of installations, for example, for introducing sheets of paper into sterile filling rooms and for the treatment of sheets of paper from contagious wards of hospitals.

TABLE LIV. BACTERICIDAL EFFECTIVENESS OF AN UV PASS-THROUGH CHAMBER

TEST ORGANISM	CONTROL PAPER		UV EXPOSED PAPER		INACTIVATION OF TEST ORGANISM, PER CENT
	Number of Paper Strips Tested	Average Number of Organisms Recovered Per Sq Inch of Paper	Number of Paper Strips Exposed	Average Number of Organisms Recovered Per Sq Inch of Paper	
Bacillus subtilis var. niger spores	20	8,300	40	0.72	99.97
Serratia marcescens	5	41,700	15	0.0	100
T-3 coliphage	5	1,160,000	15	0.0	100
Escherichia coli B/r	5	19,000	9	0.55	99.997
Escherichia coli B/r	5	19,000	26	0.60a/	99.996

a. Wash liquid or recovery plates exposed to reactivating light.

H. UV CLOTHING DISCARD RACKS

All change rooms in infectious disease laboratories should be equipped with a receptacle to hold discarded laboratory clothing. The container usually is a large canvas laundry bag held in a frame or rack. Sometimes a metal bin or a rectangular, push-type laundry hamper is provided. When a quantity of discarded clothing has collected, it should be sterilized by autoclaving before laundering.

The use of these collection devices may present a safety hazard. Although each discarded garment may contain only a low order of contamination, the method of collection has a cumulative effect. Also the container itself becomes contaminated. One contaminated article in a bag may contaminate the rest of the clothing. Clothing known to be contaminated should be immediately and separately treated. Infectious microorganisms in the discard container will be in a relatively dry form. When clothing is thrown into or removed from the container, aerosols of the agent may be produced. Certain microorganisms such as those causing Q fever are notorious for transmission via clothing. If the hamper has a large opening, the tendency is to throw the discarded clothing at the hamper. Some of this potentially contaminated clothing finds its mark, but much of it goes on the floor and is subsequently handled once again. The use of metal bins to receive discard clothing is not recommended because this necessitates the removal of the potentially contaminated clothing to another container before autoclaving.

1. The Discard Rack

A clothing discard rack (Figure 53) was designed which utilizes a protective barrier of UV radiation to isolate discarded articles in a canvas laundry bag. The unit consists essentially of two parts: (a) a metal laundry bag holder, and (b) a shielded box containing two 15-watt, hot cathode UV lamps. Both parts are mounted on a wall. The bag holder is mounted at such a height that the bottom of the canvas bag is held several inches off the floor. The UV lamps are in an inverted aluminum tray mounted above the bag holder. A curtain hangs from the lower edge of the UV box and extends down past the top edge of the laundry bag. In this manner, the UV radiation is confined, and most of the intensity is directed into the open bag.

a. Intensity Measurements

The UV box originally contained only one 15-watt UV tube. An additional tube was added because it was found that complete coverage of the bag opening was not obtained with one lamp. Intensity readings were taken of the UV radiation at the bag opening and at face level outside the unit. The average UV intensity at the opening (which is about eight inches below the lamps) taken on a horizontal plane was 653 microwatts per square centimeter. Measurements made at face level in the area surrounding the rack showed no intensities greater than 0.5 microwatts per square centimeter.

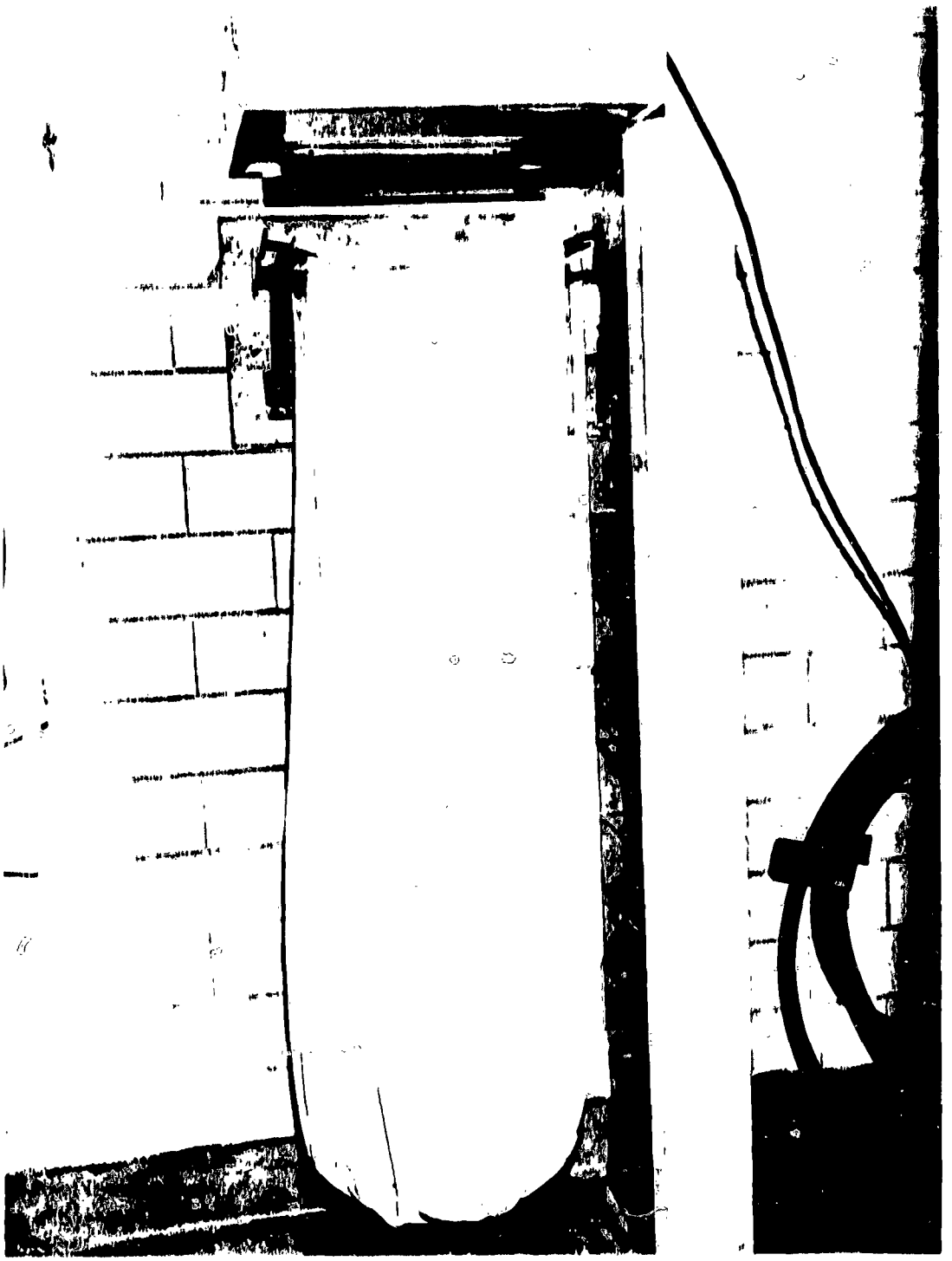


Figure 53. UV Laundry Bag Holding Device. (FD Neg B-6216)

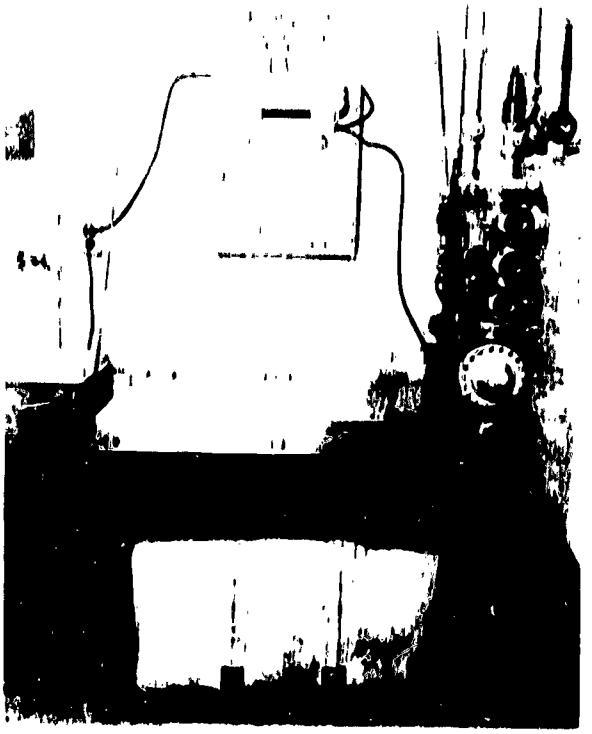


Figure 54. UV Clothing Discard Rack.
(FD Neg B-6631)

b. Results of tests

To test the effectiveness of the barrier in preventing the escape of air-borne organisms, broth cultures of S. indica were nebulized inside the laundry bag and sieve-type air samples were collected on the outside of the bag. The samples were collected during five-minute periods when the UV lamps were on and then when the lamps were off. Four sieve samplers placed around the outside of the bag were operated simultaneously. Nebulization of the test organism continued during the on and off periods. The test was repeated three times and the efficiency of the barrier calculated from the total numbers of colonies collected. Each "too numerous to count" plate was counted as 350 colonies. On this basis 6923 colonies were collected when the UV lamps were off and 34 colonies were obtained when the lamps were on. Thus, an efficiency of greater than 99.99 per cent was obtained. The actual concentration of S. indica aerosol produced in these tests was approximately 200,000 cells per cubic foot of air. This is, of course, far in excess of what might be expected under ordinary conditions.

c. Conclusions

These tests illustrate that the use of a protective barrier of UV radiation over the canvas laundry bag will eliminate the escape of almost all air-borne organisms from contaminated clothing. Another version of the clothing discard rack is shown in Figure 54.

I. PORTABLE UV FLOODLIGHT

A portable UV floodlight has been constructed and used in various ways in the infectious disease laboratories (Figure 55). The floodlight contains six, 15-watt, hot cathode UV lamps mounted in a reflector that can be adjusted to direct the radiations to any desired position. The unit is mounted on wheels and can be used to help decontaminate areas in case of accidental spills of infectious fluids. Intensity measurements of the radiation produced by this apparatus are as follows: 1.5 feet - 850 microwatts per sq cm, 3 feet - 251 microwatts per sq cm, 6 feet - 61 microwatts per sq cm of 2537A radiation. Other intensities are shown in Figure 56.

J. SAFE-T-AIRE INDUSTRIAL STERILIZER

1. Design

The Safe-T-Aire Industrial Sterilizer (Hanovia Chemical Manufacturing Company, Newark, New Jersey) is a mobile, high-pressure UV irradiator with self-contained generating plant and operating controls. The generating plant consists of a transformer, a capacitor, and a cooling fan with a safety relay to break the circuit in the event of fan failure. The plant supplies 1200 watts for a fused quartz mercury vapor lamp which is Vycor jacketed to eliminate ozone. The operating controls consist of a key operated power switch, a 1 to 15-minute adjustable starting control, and an automatic timer (15 minutes to 10 hours). These controls permit the operator to set the unit for desired operating periods and still have adequate time to leave the area before irradiation begins. Roller-bearing casters and a push-type handle permit wheeling the sterilizer from place to place. The unit is supplied for operation at 115, 220, or 440 volts.

2. Testing Methods

Bactericidal evaluation of the sterilizer was accomplished through a series of tests in which 15-milliliter quantities of Bacillus subtilis var. niger spore cultures (3×10^7 ml) and 15-milliliter quantities of Serratia indica cultures (5×10^7 ml) were nebulized in a closed room. Sieve air samples and surface samples were taken in the area at different time intervals during the period of irradiation. Control tests were run in which similar samples were taken without the sterilizer in operation. Intensity readings were taken at various distances from the lamp and at various angles off the horizontal plane from the center of the lamp (Figure 57).

3. Results

Table LV shows the results of a test in which aerosolized B. subtilis spores were exposed to the sterilizer. There was a significant reduction in the number of spores at all sampling stations, with the possible exception of No. 7. The control test, during which the sterilizer was not in operation, showed TNTC colonies at all sampling stations, at all time periods.

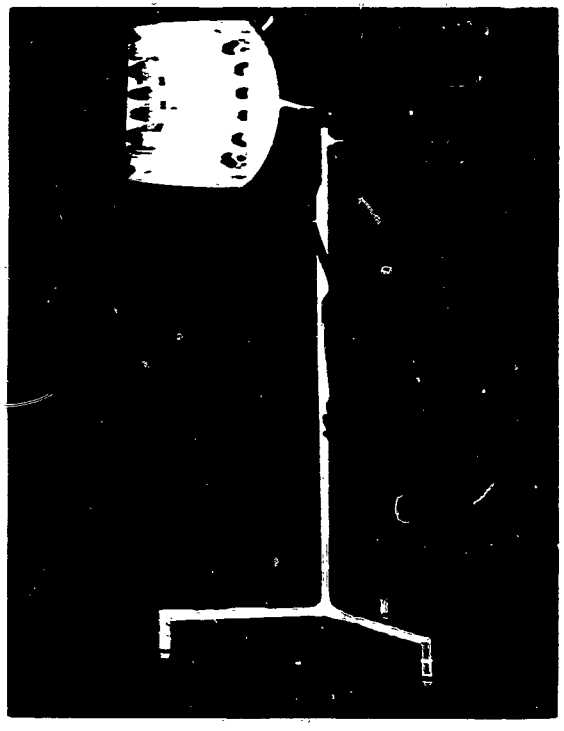


Figure 55. Portable UV Floodlight. (FD Neg B-7209)

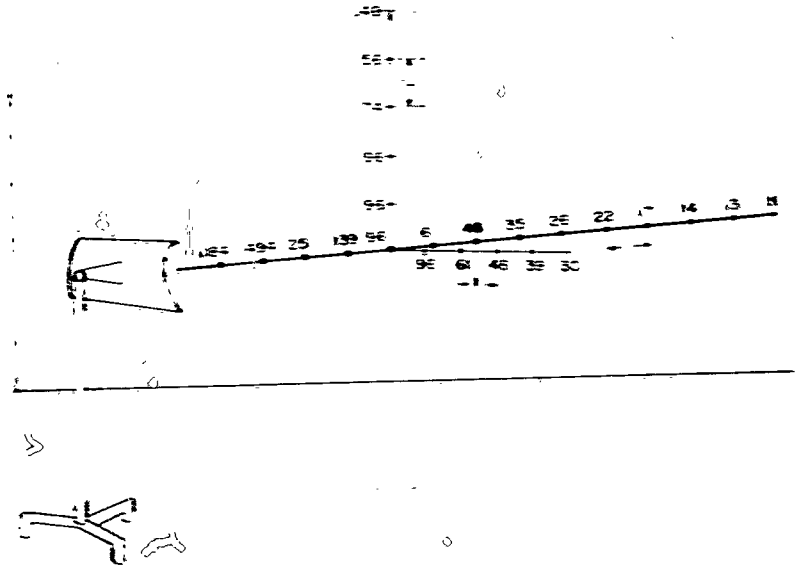


Figure 56. UV Intensities in Microwatts Per Sq Cm at Various Distances from a Portable UV Floodlight.

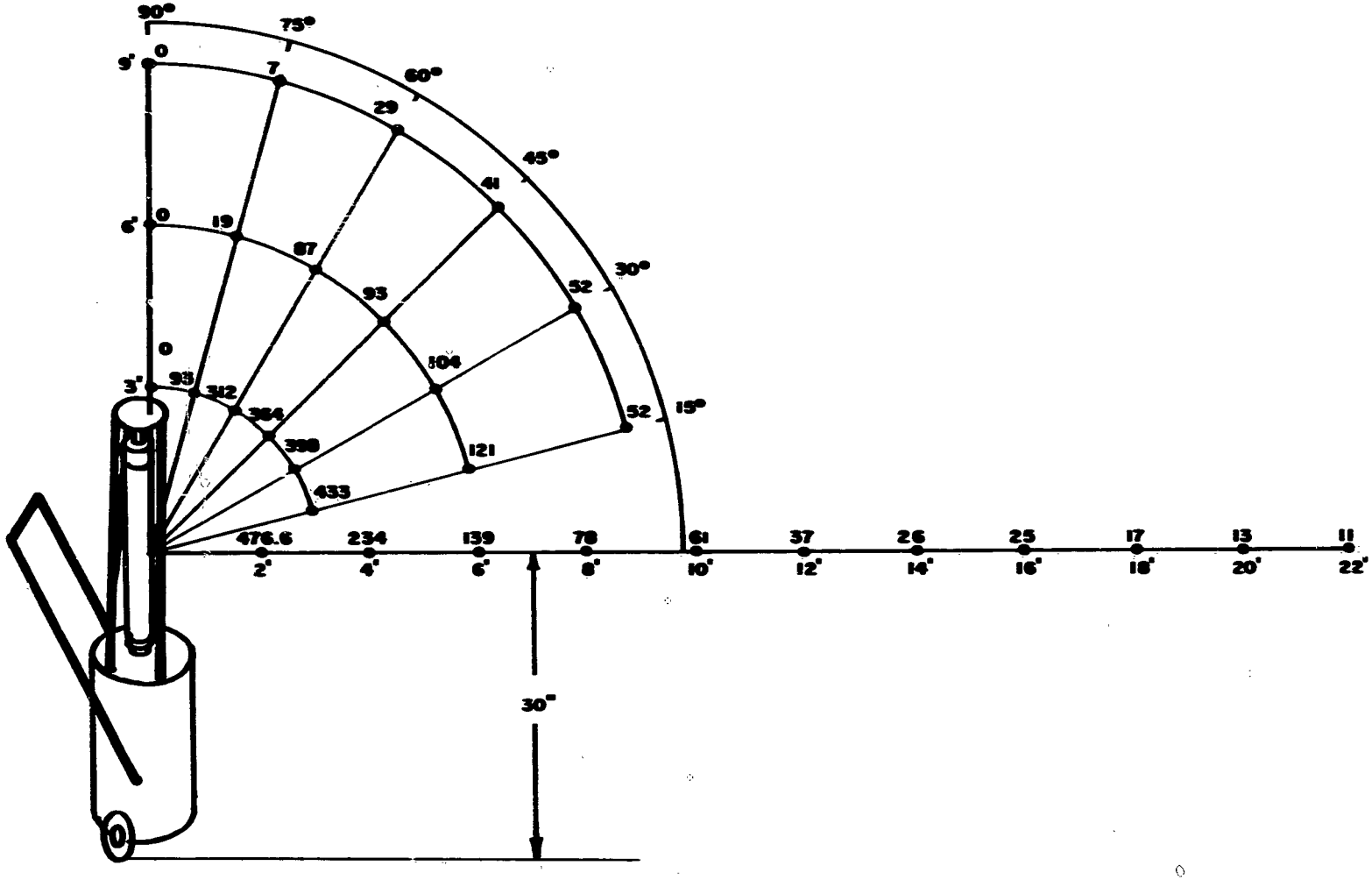


Figure 57. UV Intensities in Microwatts Per Sq Cm at Various Distances from a Portable Safe-T-Aire Sterilizer.

TABLE LV. B. SUBTILIS SPORES RECOVERED AFTER DIFFERENT EXPOSURE PERIODS TO UV RADIATION

SAMPLING STATION	DISTANCE FROM CENTER OF BULB, feet	TIME PERIODS, hours			
		0	1	2	4
1. Floor	1	TNTC	95	22	10
2. Floor	3	TNTC	134	79	56
3. Door	9	TNTC	88	63	42
4. Left Wall	7	TNTC	33	63	98
5. Right Wall	5-3/4	TNTC	5	72	0
6. Exhaust Duct - Air Sample		TNTC	TNTC	21	250
7. Shower Stall - Air Sample		TNTC	TNTC	220	TNTC
8. Floor - Air Sample		TNTC	66	276	99
9. Ceiling - Air Sample		TNTC	33	150	91

When S. indica was used, ten minutes of irradiation were sufficient to eliminate the test organism from all but one sampling station. Table LVI shows the results obtained ten minutes after nebulization, with the lamp on and with it off.

TABLE LVI. S. INDICA CELLS RECOVERED AFTER TEN-MINUTE EXPOSURE TO UV RADIATION

SAMPLING STATION	DISTANCE FROM CENTER OF LAMP, feet	UV OFF	UV ON
1. Floor	1	32	0
2. Floor	3	39	0
3. Door	9	62	0
4. Left Wall	7	181	0
5. Right Wall	5-3/4	13	0
6. Exhaust Duct - Air Sample		TNTC	0
7. Shower Stall - Air Sample		TNTC	0
8. Floor - Air Sample		TNTC	1
9. Ceiling - Air Sample		TNTC	0

4. Conclusions

The Safe-T-Aire Steriliger can effectively decontaminate the air and exposed surfaces in a small room (1740 cubic feet) contaminated with a vegetative organism and will greatly reduce contamination by spores.

K. ULTRAVIOLET RADIATION IN VENTILATED CABINETS

Ventilated cabinets used for laboratory manipulations with infectious disease organisms are often equipped with interior UV lamps (Figure 58). The radiation is usually supplied at the rate of approximately four watts of UV lamp output per cubic foot of cabinet space. Thus, generous amounts of UV energy are present to provide quick and rapid inactivation of airborne microorganisms. Because most interior cabinet surfaces are smooth, continuous, and nonporous, good decontamination of exposed surfaces is assured. The use of 2537A radiation, therefore, is helpful in decontaminating the interior of the cabinet. It must be emphasized that UV treatment should not substitute for other means of cabinet decontamination (e.g., formaldehyde or beta-propiolactone) which assure complete sterility.

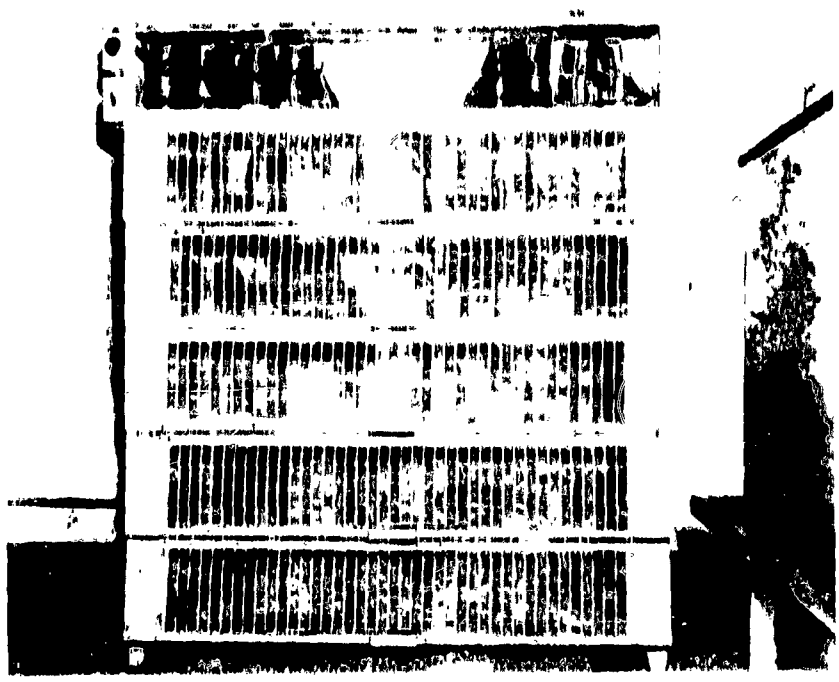
L. ULTRAVIOLET SHOE RACK

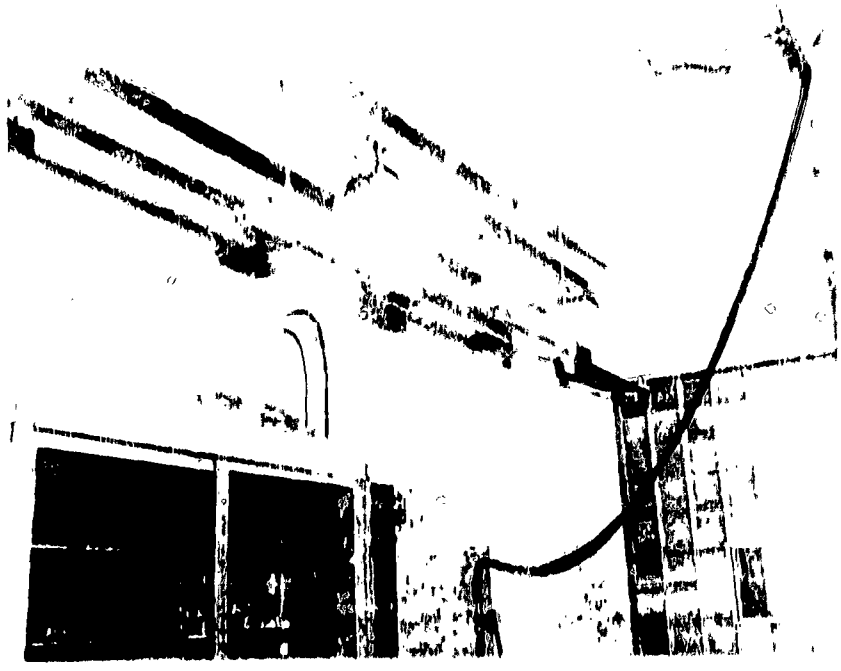
Shoes worn in the infectious disease laboratory are apt to harbor and spread pathogenic microorganisms. In many laboratories, shoes are left in the change room and are not worn outside the laboratory. It is usually recommended that the shoes be periodically decontaminated. UV lamps have been used in shoe racks as a sanitizing feature but not as a substitute for decontamination with a gas such as ethylene oxide. A shoe rack is shown in Figure 59.

M. ULTRAVIOLET RADIATION FOR DIRECT IRRADIATION OF LABORATORY ROOMS AND IN THE TREATMENT OF MOVING AIR

The practical uses of UV radiation in the ceilings of laboratory rooms and in air ducts are discussed in this section and in section XI. Figure 60 shows lamps installed in the ceilings of laboratory rooms.

Figure 58. [illegible]





XI. THE TREATMENT OF MOVING AIR WITH UV RADIATION

A. GENERAL OBSERVATIONS

Ultraviolet radiation can be employed for the treatment of air in air-conditioning or air-moving systems. However, certain limitations and problems are involved which must be thoroughly understood. These limitations must be weighed against the advantages offered by UV radiation air treatment systems.

The most important consideration is the fact that it is difficult to employ UV radiation to inactivate 100 per cent of the air-borne microorganisms in moving-air systems. For this reason the action should be termed sanitation rather than sterilization. Treatment usually involves the placement of UV lamps directly in air ducts or plenum chambers. Depending upon the volume of air handled, the duct size, temperature, and other variables, a specific number of lamps are employed in a system to give a calculated germicidal action. Installations may be designed to give 80, 90, or even 99 per cent kill of air-borne microorganisms per air passage. It is impractical to design a large system that is 100 per cent effective against all types of organisms.

Because UV air-treatment systems are not apt to produce sterile air, their usefulness in buildings where infectious materials are handled is restricted. In particular, UV radiation should not be used in recirculating systems where air exhausted from contaminated areas is drawn into a common chamber and then redistributed. If levels of contamination differ within areas, it is obvious that any system which is less than 100 per cent effective would act as a mechanism for cross-contamination. For recirculating air systems, UV treatment of the air should be used only when one room or one general area is involved. In this case continuous protection is provided because air-borne organisms are constantly being removed.

For air handling systems of the one-passage type, air sanitation by UV radiation may be used to treat the incoming air or, in certain cases, for treatment of exhaust air. If the exhaust air is to be treated, it must be ascertained that the release of a certain number of organisms to the outside atmosphere does not create a hazardous condition. The sanitation of the incoming air in infectious disease laboratories by UV radiation is often desirable. A properly designed installation should give an efficiency equal to or greater than that provided by most electrostatic or air scrubbing systems.

UV air-treatment systems have several characteristics which are not found in air-filter systems. The installation of germicidal lamps in ducts or plenum chambers does not create a noticeable increased air resistance in the system. Filters always create a resistance which must be compensated for by the use of fans or blowers with larger load capacities. UV lamps can

usually be installed in an existing air-treatment system if adequate duct space is available. The cost of an UV air-treatment system should be less than that of other systems.

The same problems and limitations inherent in all uses of germicidal UV radiation also apply to its use for sanitizing moving air. The intensity of the radiation and the exposure time must be adequate. Variations in the ambient temperature effect the operation of the lamps and consequently the germicidal action. Variations in relative humidity are, according to some, responsible for variations in the germicidal reaction. Provisions must be made for periodic testing and cleaning of the UV lamps.

B. DESIGN CRITERIA

The Slimline high-intensity lamp is recommended for moving-air-treatment systems. Lamps should be operated at a current of 420 milliamperes. Air velocities affect the lamp wall temperatures which in turn determine, in part, the total UV output. Slimline lamps are designed to operate at a slightly elevated temperature in still air so that the cooling effect of moving air improves the efficiency of the lamps.

Consideration must be given to the exact location of the lamps. In general, lamps should be placed in the ducts or plenum where the air velocity is lowest. If dust collectors or dust filters are used in the system, the lamps should always be placed after the filters. Lamps should be installed so as to provide maximum irradiation of the air. This is usually accomplished by placing the lamps at right angles to the flow of air. An opening into the duct must be provided to facilitate cleaning and testing of lamps.

A review has been made of the design criteria furnished by several UV lamp manufacturers. Various methods of calculating the number of lamps required are given, and a variety of correction factors are furnished. For a comparison of these methods, the essential information furnished by three manufacturers is outlined below.

1. Westinghouse Electric Corporation (315)

This company recommends that the average installation be designed for a 90 per cent kill of bacteria in the air. For special applications, such as in hospitals and pharmaceutical laboratories, the greatest degree of bacterial reduction possible is desired (at least 98 per cent). A curve (Figure 61) is presented to calculate the correction factor to be applied to obtain any degree of inactivation up to 98 per cent after the number of lamps for 90 per cent kill has been determined.

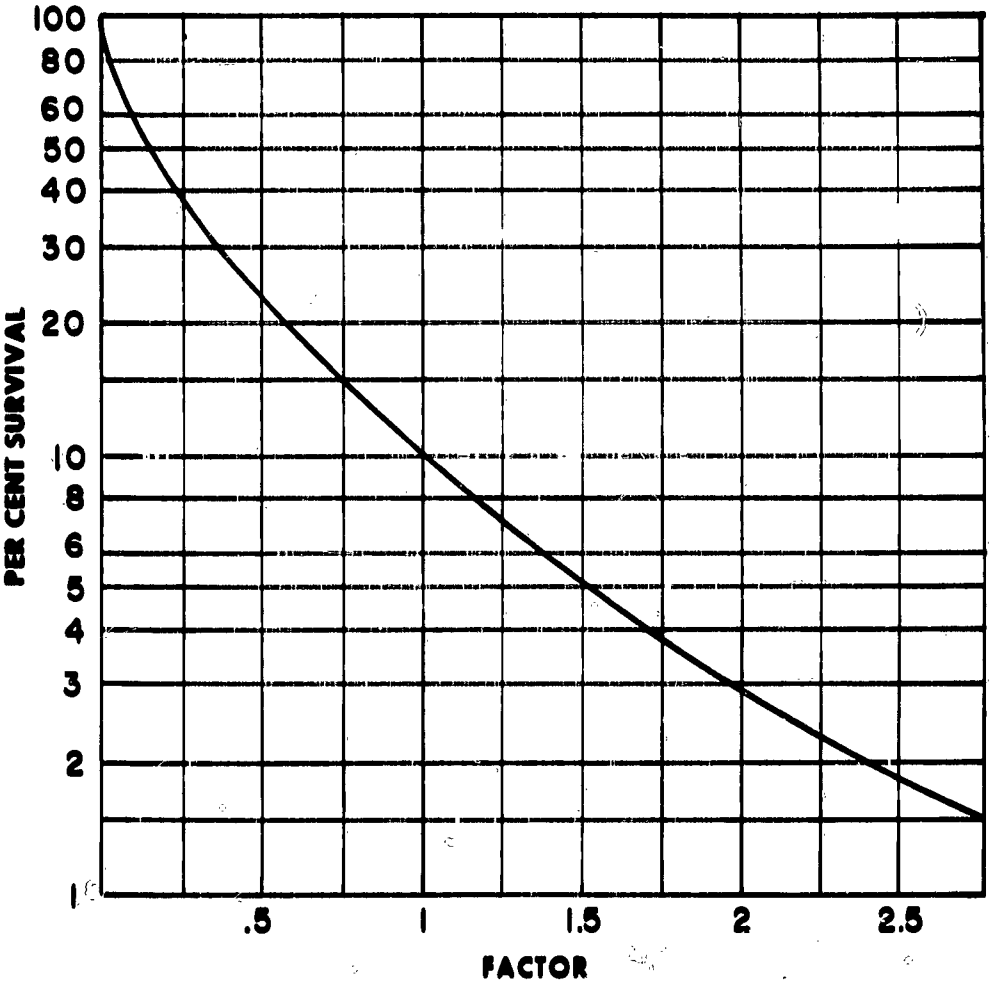


Figure 61. Curve to Calculate Lamp Requirements. To determine the number of lamps for various percentage survival first calculate for 10 per cent survival (90 per cent kill) and multiply by factor.

Where possible, lamps should be placed so that the lamp is at right angles to the flow of air and in an area where the air velocity is lowest. Lamps should be placed after filters, thus decreasing the dust and increasing lamp effectiveness. Maximum utilization of the radiation can be accomplished by painting inside duct walls with aluminum paint to increase reflectance. Lamps should be placed in a row, not less than four inches apart. If more than one row is required, the additional rows should be on four-inch centers with the lamps staggered.

The Slimline lamp is recommended. This lamp, including sockets, is approximately 36 inches long. Calculations are made on the basis that one dimension of the duct is 36 inches, or a multiple of 36 inches. Each installation should be equipped with a service door and inspection window in the duct. The door should be equipped with an electrical circuit breaker to turn off the lamps automatically when the door is opened.

To calculate the lamp requirements for any given installation, the following information is required: (a) width (W) and length (L) of the irradiation chamber (Height is the dimension parallel to the lamps and is always 36 inches or a multiple thereof.), (b) volume of air handled (CFM), (c) air velocity (LFM), and (d) temperature of air (°F).

The first step is to calculate the volume of air disinfected by one watt of UV (VDW). The duct width (W) and length (L) is used in Figure 62 to obtain this value. If the duct height is greater than 36 inches, and two or more banks of lamps in the same vertical plane are used, a correction factor must be applied to the VDW value. The correction factor is found in Figure 63 and has the net effect of lowering the number of lamps required.

The next step is to calculate the corrected lamp output (G36T6 Slimline Steriiamp at 420 ma). Using the values of the minimum ambient temperature in the duct and the air velocity, the UV output in watts, is found in Figure 64.

Finally the number of lamps required is found by the following formula:

$$\text{Number of Slimline lamps required} = \frac{\text{CFM}}{\text{VDW} \times \text{corrected lamp output}}$$

This method gives the number of lamps required for a 90 per cent destruction of bacteria. Higher rates of disinfection are obtained by multiplying by the correction factors found in Figure 61.

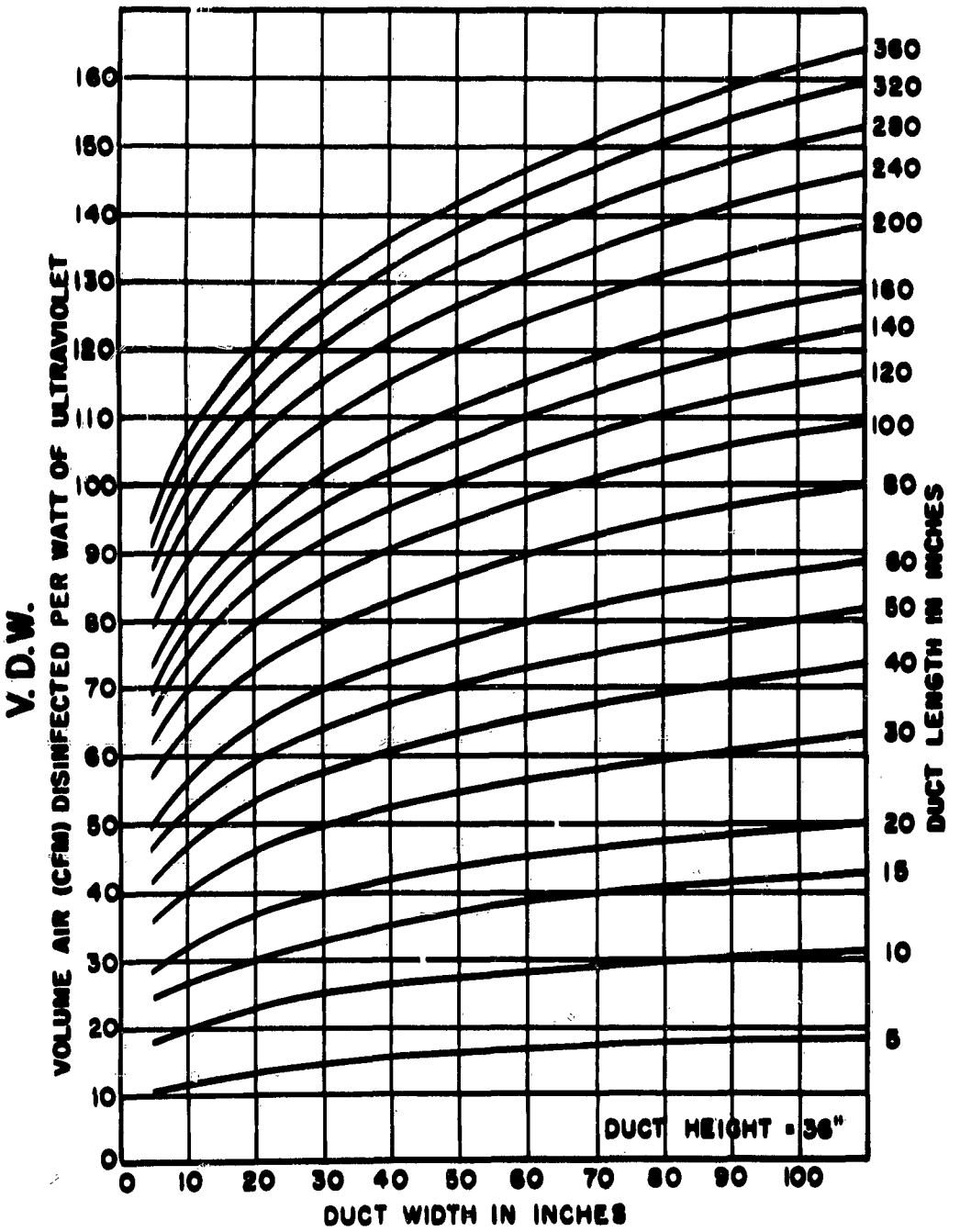


Figure 62. Curves Showing V.D.W. Values.

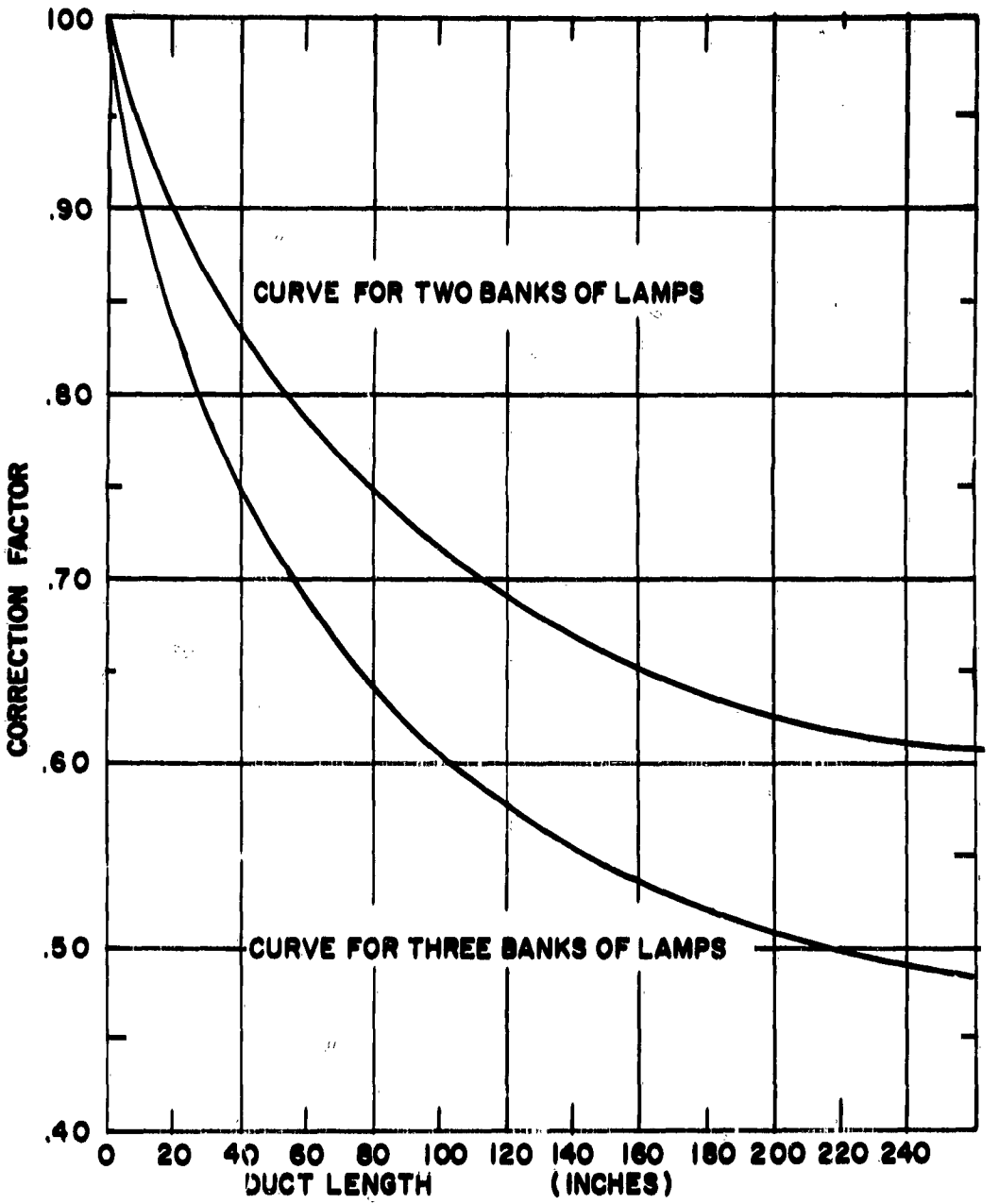


Figure 63. Correction Curve for Two or Three Banks of Lamps.

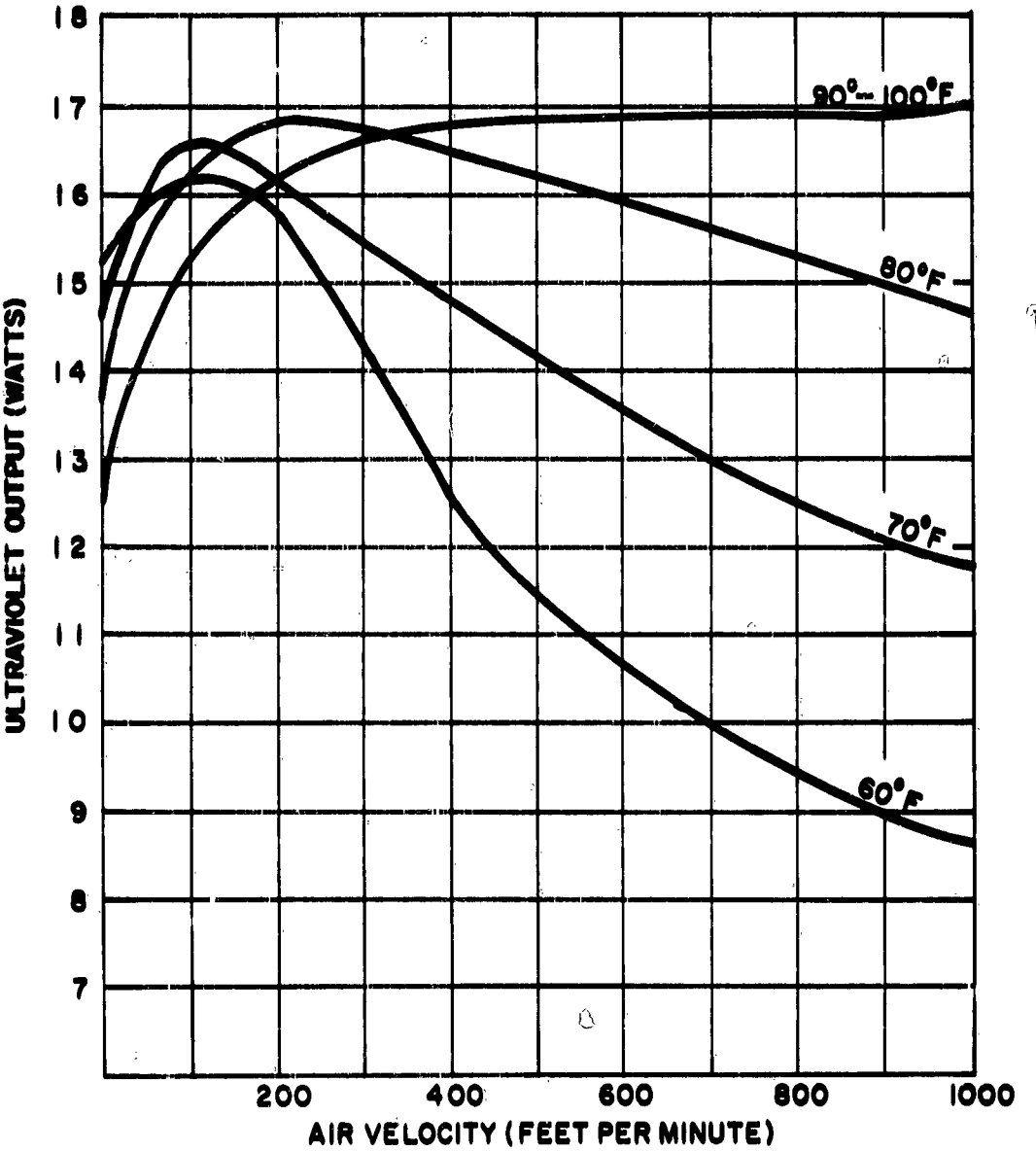


Figure 64. UV Output as Affected by Temperature and Air Flow. G36T6 Slimline Sterilamp at 420 ma.

2. General Electric Company (97, 98)

General Electric Company recommends that lamps be placed lengthwise on the duct walls on four- to five-inch centers and grouped in the center half of the duct walls. The duct walls should be made of polished chromium plate or aluminum. Arrangements must be made for cleaning the lamps. Hinged panels on the sides or bottom of the duct should be provided; the lamps may be mounted on these panels if necessary.

A theoretical 99 per cent disinfection of air is recommended, and installation data are calculated on this basis. The total UV watts (UVW) required in nonreflective ducts for a theoretical 99 per cent disinfection is calculated from the following formula:

$$UVW = \frac{CFM}{3 \times d}$$

where d = the lesser duct dimension in inches, provided it is not exceeded by more than 50 per cent by the larger dimension. If the duct has one dimension which is two or more times as great as the other, the formula can be used by subdividing the duct and air capacity so that the dimensions are as required.

The total UV watts required (UVW) is divided by the average output of the type of lamps to be used to obtain the number of lamps required. The conditions specified are 80°F, relative humidity of 60 per cent or below and nonreflective ducts. The following correction factors, if applicable, should be applied:

Condition	Increase the Number of Lamps Used by
Temperature of 50°F	10%
Temperature of 100°F	10%
Temperature of 40°F	20%
Temperature of 110°F	20%
Temperature of 35°F	30%
Relative humidity of 70%	50%
Relative humidity of 80%	65%
Relative humidity of 90%	75%

If the duct walls have a reflectance of 75 per cent, a theoretical 99 per cent disinfection of air can be obtained with one-half the calculated number of lamps. For nonreflective duct walls, a reduction of 90 per cent can be obtained with one-half the number of lamps required for 99 per cent disinfection.

Figure 65 is supplied by General Electric Company to assist in calculating duct lamp requirements.

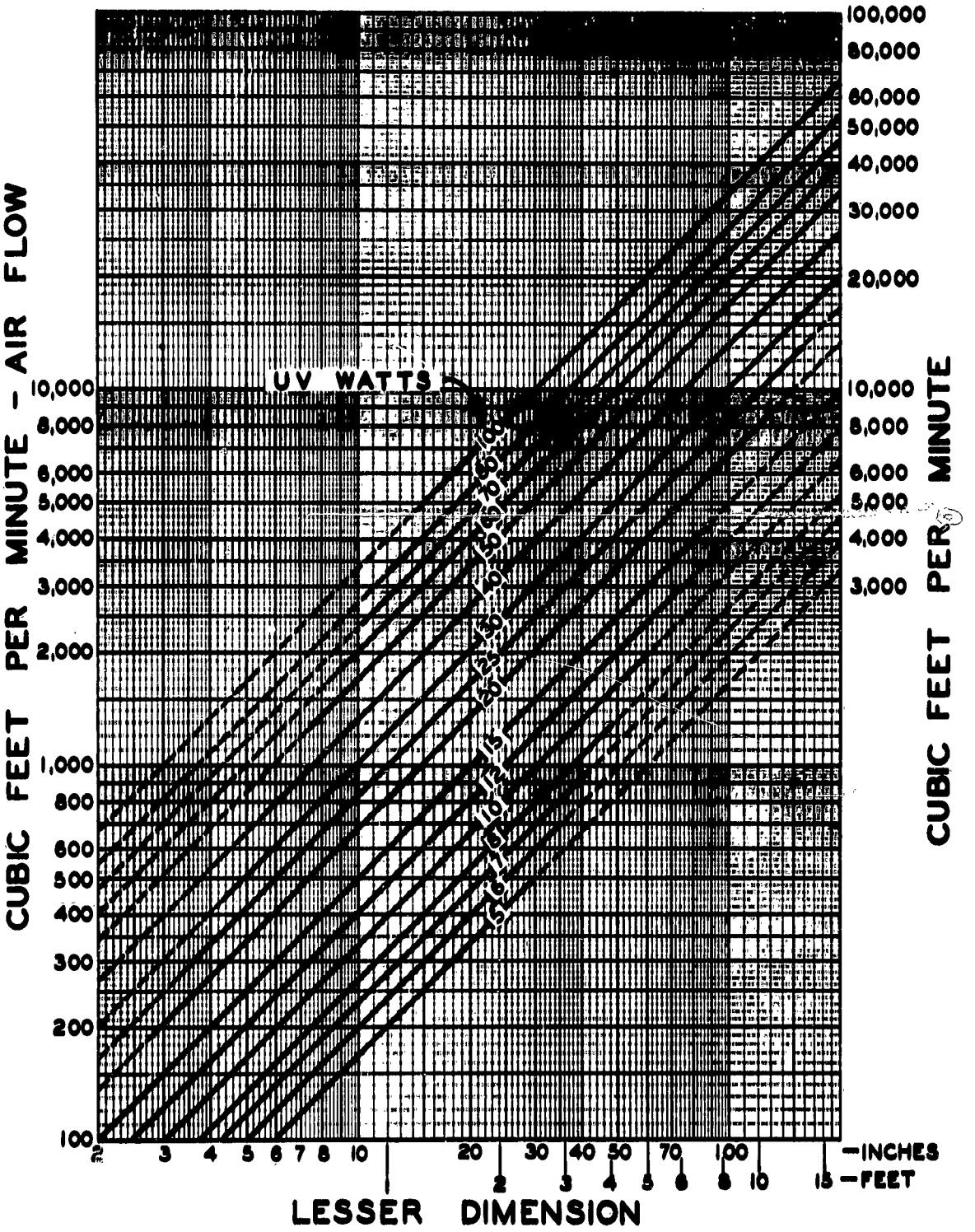


Figure 65. Theoretical 99 Per Cent Disinfection of Air at 80°F in Nonreflective Ducts.

3. Hanovia Chemical and Manufacturing Company (116)

Information supplied by the Hanovia Company states that "bacteriological tests in rooms have shown that each person in a room which contains a number of persons must receive 500 cubic feet of complete air change of pure air per minute if the air he breathes is to be maintained in a sanitary condition." The "lethe" is equivalent to one complete air change by pure air or a reduction of 63.2 per cent in the bacterial content of the air in an unoccupied room. One lethe of disinfection results from uniform radiation of wave length 2537A angstroms at an intensity of 35×10^{-6} watt-hour per square foot in a room in which the relative humidity is below 50 per cent. It was therefore postulated that each person in a room needs 500 cubic-foot lethes per minute or 30,000 cubic-foot lethes per hour and that UV radiation may be used to provide equivalent sanitary ventilation. This basis is used to devise formulas for calculating lamp requirements.

For each installation the following data should be obtained:

V = volume of air duct, cubic feet
C = volume of room, cubic feet
N = maximum number of persons in room
R = radiation length factor, average distance between the lamp centers and the nearest duct wall
Duct dimensions, feet
Air flow, cubic feet per hour
Relative humidity, normally taken as 50 per cent

Step 1 - Calculate the requirements of the room in terms of lethes per hour. Lethes per hour =

$$\frac{N \times 30{,}000 \text{ cu ft lethes/hr/person}}{C}$$

Step 2 - Calculate the maximum available lethes per hour through the duct. Maximum lethes per hour through the duct =

$$\frac{\text{air flow, cu ft per hour}}{C} \quad \text{or}$$

$$\frac{\text{air flow, cu ft per hour} \times \text{lethes per hour}}{N \times 30{,}000 \text{ cu ft per hour per person}}$$

Step 3 - Calculate Id, the theoretical average intensity in the duct in terms of watts per square foot.

Id = lethes per hour through duct $\times 35 \times 10^{-6}$ watts per sq ft

Hanovia Company recommends that the energy value (35×10^{-6}) be changed according to Figure 66 if higher humidities are present.

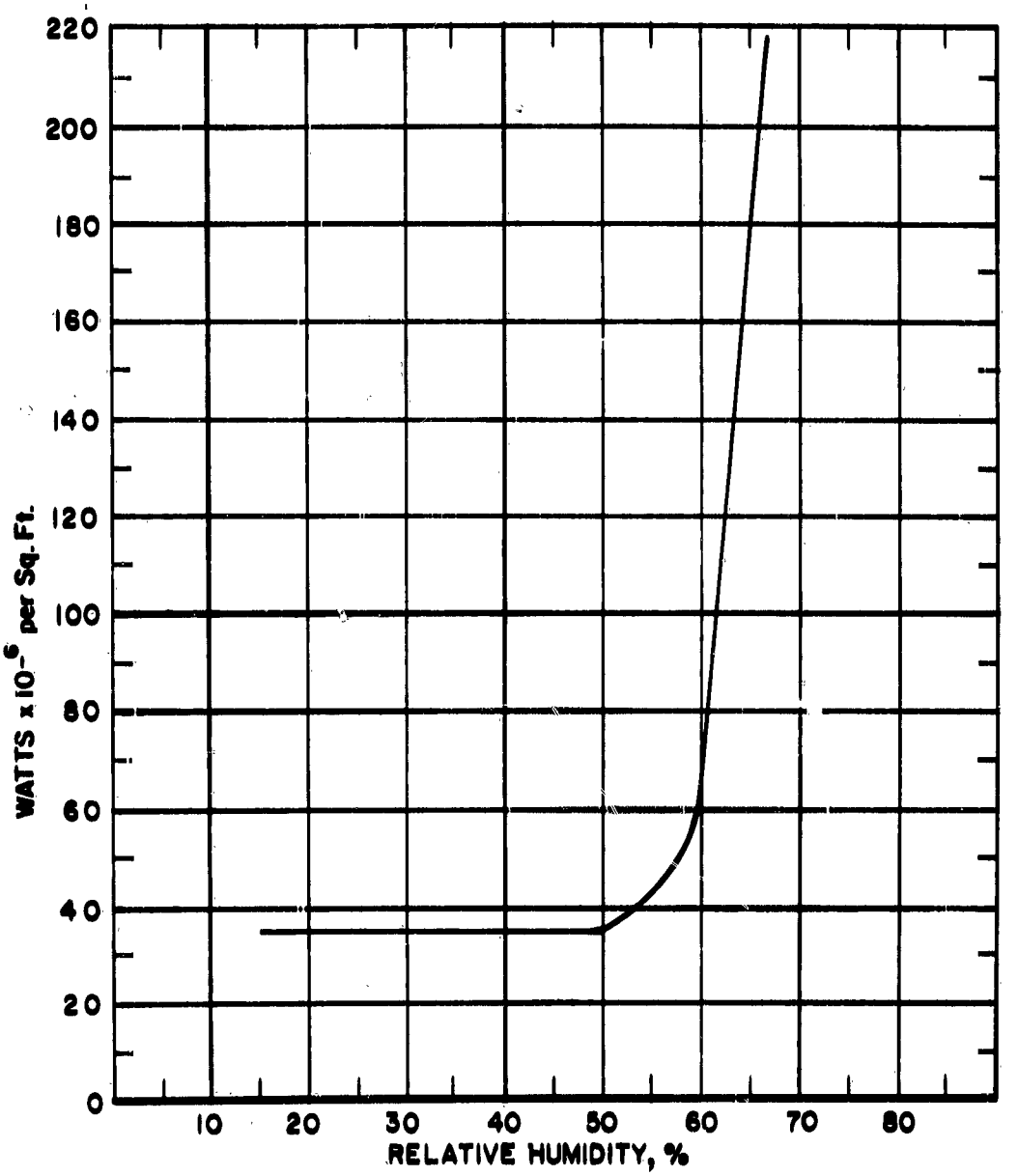


Figure 66. Variation of Radiation 2537A in Lethe Per Hour with Changes in Relative Humidity.

Step 4 - Calculate Ed, the theoretical total flux in watts of 2537A radiation.

$$Ed = Id\ V^2/3$$

Step 5 - Calculate the number of lamps required

$$\text{Number of lamps} = \frac{Ed \times 4}{LR}$$

4 is the "efficiency factor for lamps in ducts"
L is the lamp rating of lamp to be used
R is the radiation length factor obtained from Figure 67

C. APPLICATIONS

The methods of application of radiation in air ducts have been taken nearly verbatim from the literature of the various manufacturers. A comparison of the calculations given in their literature shows that sometimes there are differences in the amount of radiation required to accomplish a given disinfection. Although it is questionable, Rentschler and James* (142), reported that air-borne pathogenic bacteria could be inactivated with one-tenth the radiation necessary to inactivate the same bacteria on agar plates. These differences are probably due to the fact that each of the investigators used different types of bacterial air samplers, different rates of air flow, humidities, microorganisms, and probably different methods of reasoning for their calculations. Some of these variations have been discussed by Nagy (218). It would appear that more tests would be necessary to standardize this method of disinfecting air.

Unfortunately there are few adequate evaluations in the literature on the sanitizing efficiency of moving-air systems provided with UV lamps. However literature available from lamp manufacturers illustrate some of the systems which have been used.

It has been pointed out (314) that UV lamps can be installed in small air-conditioning units to provide a reduction in air-borne contamination equal to the reduction obtained in larger systems. Lamps were installed in a unit which supplied air to six operating rooms in a dental office. Eight 17-watt tubes were mounted in the incoming air duct. Tests made before installation showed that cross-infection of air-borne particles between different rooms occurred. Later tests showed that the installation of UV radiation greatly reduced the incidence of cross-infection between rooms.

Tests of an air-conditioning system of an auditorium have been reported by Rentschler and Nagy (245). The system was designed to circulate 5800 cubic feet per minute of air. Different numbers of lamps were placed in a

* Cited in Hibben et al.

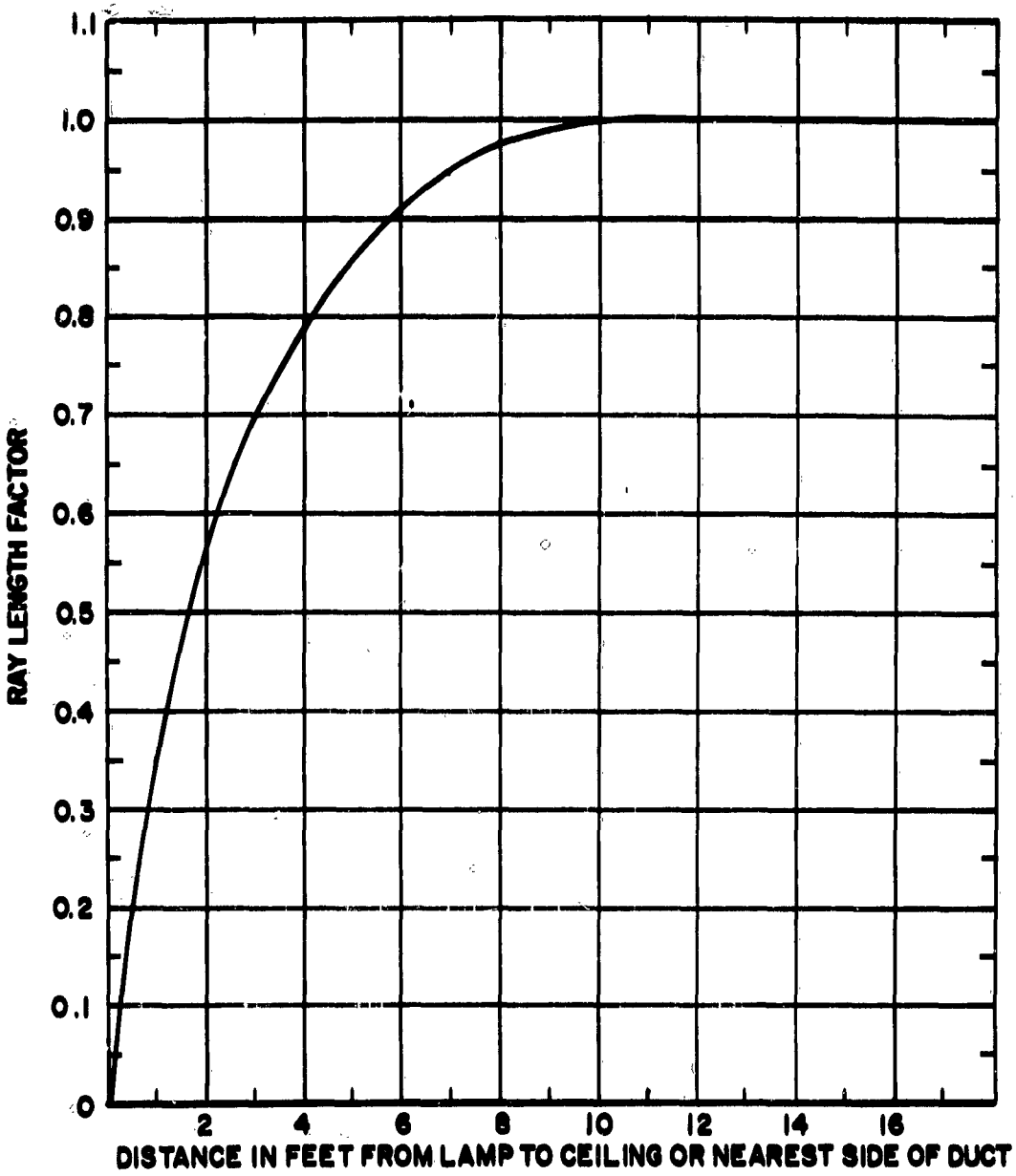


Figure 67. Ray Length Factors for Various Distances Between Lamps and Ceiling, or Between Lamps and Nearest Duct Wall.

75 by 30 inch irradiation chamber and tests of efficiency were made using sprayed cultures of E. coli. Air samples were taken at various points while the lamps were off and again with the lamps on. The use of 40 cold cathode lamps gave an average reduction of air-borne E. coli of 99.5 per cent, while 32 lamps gave a reduction of 98.2 per cent. Rentschler drew the conclusions that (a) there is no difference in the bactericidal effect of UV radiation over a range of relative humidity between 35 and 95 per cent and no difference over the normal range of temperatures usually found in air-conditioning systems, (b) curves can be prepared to estimate the number of lamps required for any particular system, and (c) UV radiation has the particular advantage that nothing is added or removed from the air.

Successful use of UV lamps in the air systems of several pharmaceutical plants has been reported (202,314).

D. SAFETY REQUIREMENTS FOR UV INSTALLATIONS

Most of the essential industrial safety considerations involved in UV installations for sanitizing moving air have been outlined by Dennington (62). The information listed below has been taken from his report.

1. Fire and Smoke Hazards

Fire underwriters require that air ducts be constructed, equipped, and maintained in such a manner that there is no chance of damage by fire and smoke originating within the duct. When ordinary electric lights are mounted in air ducts, they must be equipped with vapor proof covers because the temperature of the heated filaments, in case of lamp breakage, can cause ignition of dust or other materials. The temperatures at the warmest points on UV lamps used in air-conditioning ducts (cold cathode or Slimline lamps) are not high enough to be considered a fire hazard. With an ambient air temperature of 70°F, the average temperature directly over the active discharge portion of the cold cathode lamp is 122°F. At the warmest point over the cold cathode lamp, directly over the electrode, the temperature does not exceed 214°F.

In order to allow for the installation of UV lamps, paragraph 181 of the Standards of the National Board of Fire Underwriters for the Installation of Air Conditioning, Warm Air Heating, Air Cooling and Ventilating Systems, has been modified to read as follows:

> "Electrical wiring and equipment shall be installed in accordance with the National Electrical Code. Lamps within the enclosure of the conditioning system shall be enclosed in fixtures of the marine (vapor-tight) type, except that germicidal lamps of a type not using filament and which operate at relatively low exposed surface temperatures, need not be so enclosed."

2. Wiring

In an UV installation, the only material which can be classified as combustible is the insulation on the wires connecting the various lamp receptacles. Current regulating transformers should be placed outside the duct. Insulated wires should be GTO-5 gas tube sign cable or the equivalent. Wires should be placed in enclosed raceways wherever possible as a protection against abrasion.

3. Windows

Glass windows are usually provided for inspection purposes. Wired glass is required for mechanical strength and resistance to shattering. Underwriters' regulations specify that no individual window shall exceed 720 square inches in area. The glass must be "...supported by metal troughs or clips of not less than 24 U.S.G., one-half inch in width, overlapping the glass one-half inch and spaced not more than six inches along each edge of the glass nor closer than 1½ inches from a corner. Windows must be fitted substantially airtight to prevent leakage of air from the duct. However, if gaskets are used, they must be of felt or synthetic material not affected by ozone...."

4. Doors

Service doors must be provided to permit the testing and replacing of UV lamps. It is usually necessary to provide interlocking switches on service doors which will open the primary circuit supplying the transformers. This system is recommended to provide safety protection from high intensity UV radiation and from possible contact with high voltage wires. If interlocking service door switches are used, an alternate method must also be provided to allow for the testing of lamps. For this, the circuits may be arranged so that one or two transformers may be put into operation individually.

5. Circuits and Lamp Mountings

Underwriters' regulations specify that, "Primary circuits shall be so arranged that no group of transformers on any one circuit shall require in excess of 15 amperes. It is desirable to keep the number of transformers per circuit eight or less to permit greater flexibility in operating and testing."

There may be instances where the dimension of the air duct is a few inches shorter than the lamp to be installed. In this case, the lamps may pass through holes in the wall of the duct, and the electrode receptacles may be supported on brackets outside the duct. For this, a tight cover must be provided over the receptacles to prevent leakage. Transformers should be enclosed singly or in groups in metal enclosures of material not less than No. 24 U.S.G. in thickness and mounted on the outside top or side of the air duct.

E. EXPERIMENTS WITH UV RADIATION IN AIR-HANDLING SYSTEMS

1. Large-Volume Air System

An UV installation designed to treat the air in a recirculating air-conditioning system in an infectious disease building has been tested. The system handles air from one large room and recirculates and conditions it in large ducts and plenum chambers which are housed in an adjoining utility room. UV radiation was chosen as the best means of treating this air because the design of the system was such that the resistance afforded by bacterial filters would seriously reduce the capacity of the system.

a. Ultraviolet Installation

UV lamps were installed at the opening of the air return duct located on the ground level of the building. The duct opening at this point measures approximately 128 by 102 inches, or 90.66 square feet. After the installation of UV lamps and suitable dust filters, the average air flow through the duct return opening was 42,400 cubic feet per minute. Sixty UV, high-intensity Slimline lamps were mounted in the duct opening. Thirty lamps were placed vertically in each of two banks (Figure 68). Each lamp was operated at a current of 420 milliamperes. To prevent the radiation from creating a hazard in the adjacent working area, it was necessary to install metal dust filters around the lamps. Tests showed that the level of radiation in the working area was thus reduced below the maximum allowable concentration. The distance from each lamp to the dust filters was about 41 inches. In the other direction (into the duct), the radiations could be effective over a distance of from 5 to 15 feet. Westinghouse data were used as a guide for the design, which was made on the basis of an estimated 99 per cent reduction of air-borne microorganisms per unit of air passing the lamps.

UV lamps, when new, produce abnormally high amounts of radiant energy. Accordingly, the lamps were allowed to burn for approximately six weeks before testing was begun. Intensity measurements were made of all lamps with an SM 600 UV meter. The average intensity per lamp at one meter was 98.6 microwatts per square centimeter, with a range of 85 to 117 microwatts per square centimeter. All lamps were cleaned before each test.

b. Test Methods

The tests were conducted in the following manner: A culture of S. indica was sprayed continuously into the air in the large room (125,000 cubic feet), approximately 15 feet from the duct intake opening, for a period of 40 minutes. The culture was sprayed at the rate of 20 to 25 milliliters per minute. Approximately one liter of culture was used for each test. Aerosol recovery samples were taken with sieve air samplers from sampler adapter lines installed in the air-handling ducts on the downstream side of the UV lamps. Usually one sieve sampler was located in the large test room several feet from the spray device. During the first 20 minutes

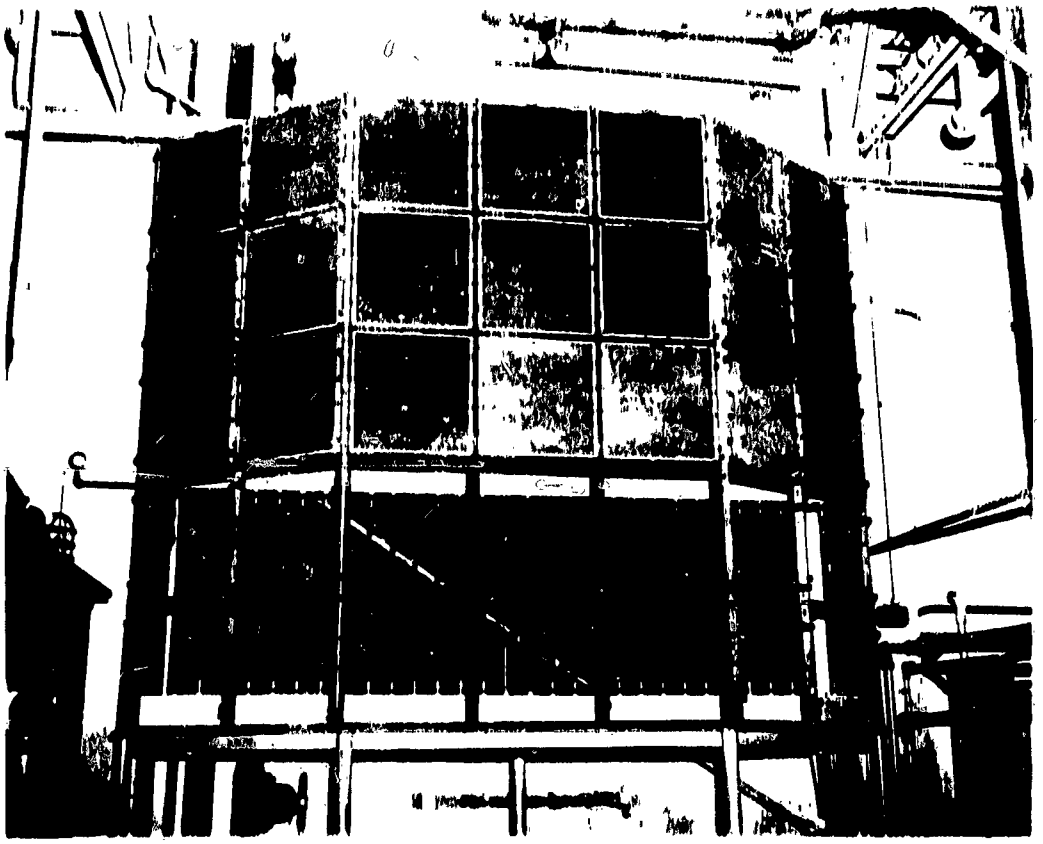


Figure 62. Large Volume UV Air Sterilizer. (PD Neg. B-6632)

of each test the UV lamps were on and for the last half, samples were taken with the lamps off. Control samples also were taken before each test. Aerosol concentrations were recorded as organisms per cubic foot of air.

c. Results

In Test 1 a 24-hour broth culture of S. indica was sprayed. The results are shown in Table LVII. All control air samples taken before the test were negative for S. indica. Aerosol concentrations during the off period averaged 49 organisms per cubic foot of air. No test organisms were recovered during the on period, indicating a 100 per cent efficiency at this aerosol concentration. During the test, the dust filters were in place.

TABLE LVII. RECOVERY OF S. INDICA IN AN AIR-CONDITIONING DUCT (TEST 1)[a]

TIME, minutes	NUMBER OF S. INDICA CELLS RECOVERED PER CUBIC FOOT			
	UV Lights On		UV Lights Off	
	Location A	Location B	Location A	Location B
0-5	0	0	63.6	49.0
5-10	0	0	50.0	43.6
10-15	0	0	49.0	40.8
15-20	0	0	---[b]	---[b]

a. Control air samples taken immediately prior to the test were negative for S. indica.
b. Not tested.

In Test 2 the procedures used were the same as in Test 1 except that the dust filters were removed during the test. Removal of the filters allowed higher aerosol concentration to enter the air duct. A 36-hour broth culture having a count of 36×10^7 S. indica cells per milliliter was sprayed. The results are given in Table LVIII. The UV radiation again was 100 per cent effective in inactivating the S. indica aerosol, although in this case, the samples taken with the UV off showed colonies too numerous to count. The aerosol concentration was estimated to be approximately 200 particles per cubic foot.

TABLE LVIII. RECOVERY OF S. INDICA IN AN AIR-CONDITIONING DUCT (TEST 2)[a]

TIME, minutes	NUMBER OF S. INDICA CELLS RECOVERED PER CUBIC FOOT			
	UV Lights On		UV Lights Off	
	Location A	Location B	Location A	Location B
0-5	0	0	TNTC	TNTC
5-10	0	0	TNTC	TNTC
10-15	0	0	TNTC	TNTC
15-20	0	0	TNTC	TNTC

a. Control air samples taken immediately prior to the test were negative for S. indica.

During this test, samples also were taken at the point where the spray was generated, which was approximately 15 feet from the duct intake opening. With the dust filters removed from the opening, the UV radiation emanating from the duct apparently was effective even in the area around the spray position. Air samples taken at the spray position while the duct UV lights were off contained S. indica cells too numerous to count. Air samples taken at the spray position while the duct UV lights were on contained only 1 to 10 S. indica cells per cubic foot.

Sieve samplers also were used to sample the air in the adjoining utility room during this test. During the 20 minutes when the lamps were on no test organisms were recovered, but during the off period three out of eight samples showed recovery of S. indica. This result indicated that the plastic coating on the ducts in the utility room was not aerosol-tight and that the use of UV radiation prevented escape of test organisms into the utility room.

In Test 3 samples were taken at four locations in the air duct. Samplers were operated for only one minute each in order to obtain a countable number of colonies on the agar plates. The dust filters were removed for the test. One liter of S. indica broth culture (9×10^7 cells per ml) was sprayed during the 40-minute period. The results are shown in Table LIX. Except for one colony appearing on the sample taken at Station D during the third sample period, complete inactivation was obtained of aerosols containing as many as 196 S. indica cells per cubic foot.

TABLE LIX. RECOVERY OF S. INDICA IN THE AIR-CONDITIONING DUCT (TEST 3)[a]

SAMPLE NUMBER	NUMBER OF S. INDICA CELLS RECOVERED PER CUBIC FOOT							
	UV Lights On				UV Lights Off			
	Station				Station			
	A	B	C	D	A	B	C	D
1	0	0	0	0	25	187	41	28
2	0	0	0	0	12	90	22	12
3	0	0	0	1	11	111	32	19
4	0	0	0	0	15	64	42	18
5	0	0	0	0	12	137	99	34
6	0	0	0	0	17	163	88	36
7	0	0	0	0	25	196	93	55
8	0	0	0	0	30	136	81	38

a. Control air samples taken immediately prior to the test were negative for S. indica.

d. Conclusions

From these tests it was concluded that the installation was 100 per cent effective in inactivating air-borne S. indica in concentrations as high as 196 cells per cubic foot, when the air system was handling approximately 42,400 cubic feet per minute of air.

2. Room-Type Air-Conditioning Units

In infectious disease laboratories room-type air-conditioning units are sometimes used to provide local temperature control. Since these units often recycle the air, sanitation of such air may be required for the safety of workers.

In the following experiments, the utilization of UV lamps installed in a room air-conditioner was evaluated to determine whether there would be a resultant reduction in bacterial contamination of room air without excessive radiation exposure of animals or laboratory workers (118).

a. Methods

The window-type room air-conditioner (Model 7975D4) used was a 3/4 HP unit of a standard commercial design manufactured by the United States Air Conditioning Corporation, Minneapolis, Minnesota (Figure 69).

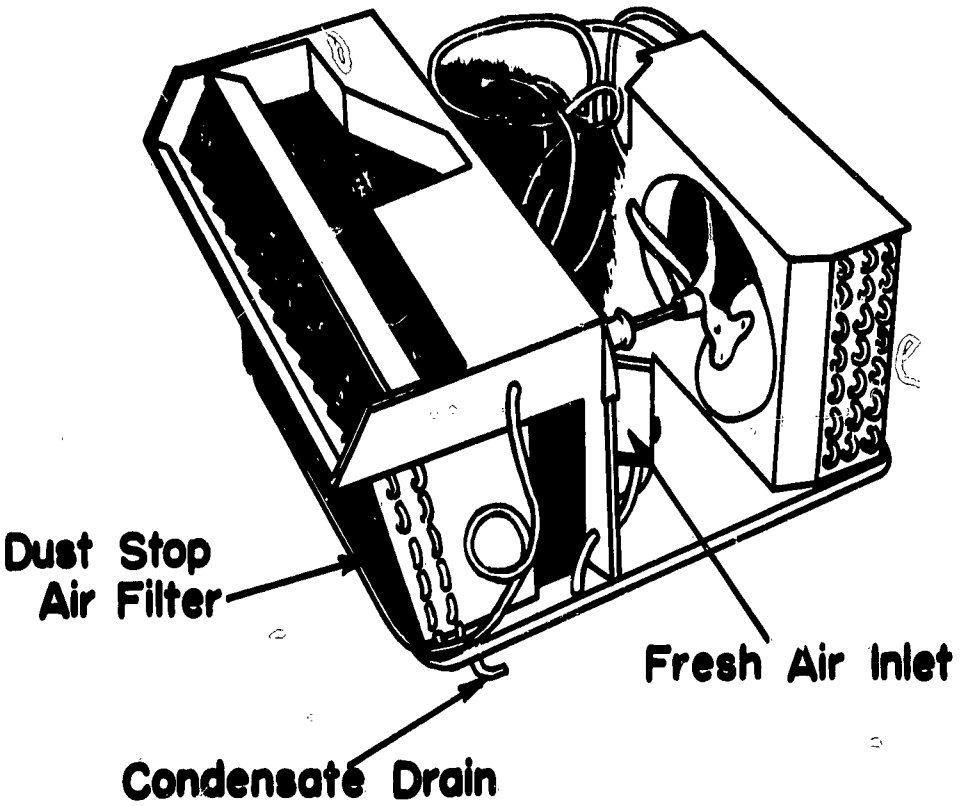


Figure 69. Window-Type Room Air Conditioner.

It had air-circulating capacity of 310 cubic feet per minute, an exhaust air capacity of 150 cubic feet per minute and a fresh air capacity of 60 cubic feet per minute. For the tests the exhaust damper was closed and the 310 cubic feet per minute of air consisted of 30 cubic feet per minute of outside air and 280 cubic feet per minute of room air. In this type of conditioner, condensate resulting from dehumidification of room air is sprayed against hot condenser coils by a slinger ring on the condenser fan and disposed of in vapor form. However, the system was altered for test purposes and the condensate was collected and sampled for bacteria. The unit was equipped with a "dust stop" to filter out large dust particles from the return air. The conditioner was installed in a laboratory room 10 by 12 by 9 feet. The test room, air conditioner, and additional apparatus for testing the air conditioner are shown in Figure 70. Cultures of S. indica were atomized into the room by a Chicago-type atomizer. A propeller fan mixed the aerosol with the room air.

Figure 71 shows the air conditioner adapted for this study. The duct was equipped to hold one or two UV lamps and painted aluminum to increase reflectance. Supply air and UV-absorbing ducts were added for sampling purposes. The duct was coated with a zinc oxide, oil-base paint for maximum absorption of 2537A radiation so that return air samplers placed in this duct would not be affected by radiation from the UV lamps. Air samplers placed in the supply duct assured a sample of supply air only. Wet and dry bulb thermometers located in the UV-absorbent duct recorded the relative humidity and temperature of the room air returning to the air conditioner.

UV irradiation was supplied by G36T6-L Slimline lamps. The intensity of the lamps was measured in microwatts per square centimeter at one meter by a Westinghouse SM-600 meter. The temperature and velocity of the air were measured because these factors affect UV lamp intensity. Velocity across the lamps during intensity measurements was approximately ten feet per minute, but during all tests, the velocity in the duct was 200 feet per minute. The temperature varied from 70.5° to 75.5°F. Before any measurements were taken, the lamps were cleaned with an alcohol dampened cloth and allowed to operate for approximately 15 minutes to warm.

Air samples were collected with liquid impinger samplers in the absorbent duct to determine the concentration of the test aerosol returning to the air conditioner. Samples of supply air were collected in the supply duct with sieve-air samplers operated at the rate of one cubic foot per minute.

Penetration of air-borne organisms through the air conditioner without utilization of UV lamps was studied in the first two tests. The penetrations were 35 per cent and 45 per cent, respectively. Partial removal of the test organism from the air stream was attributed to impingement on the dust-stop filter and on internal surfaces of the air conditioner and duct work.

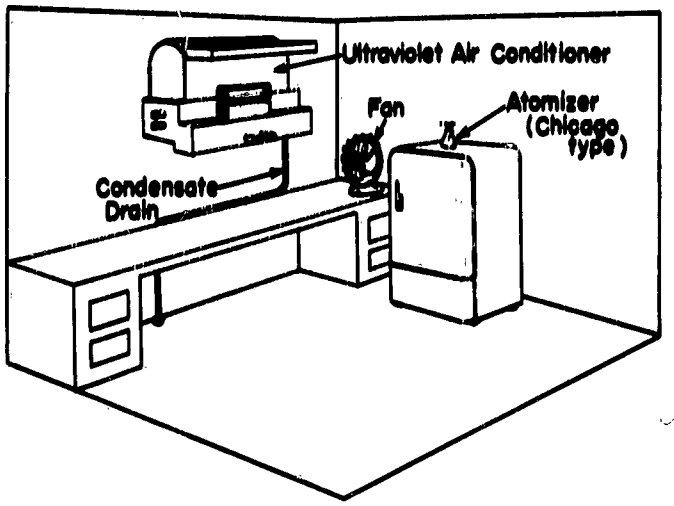


Figure 70. Air Conditioner Test Room.

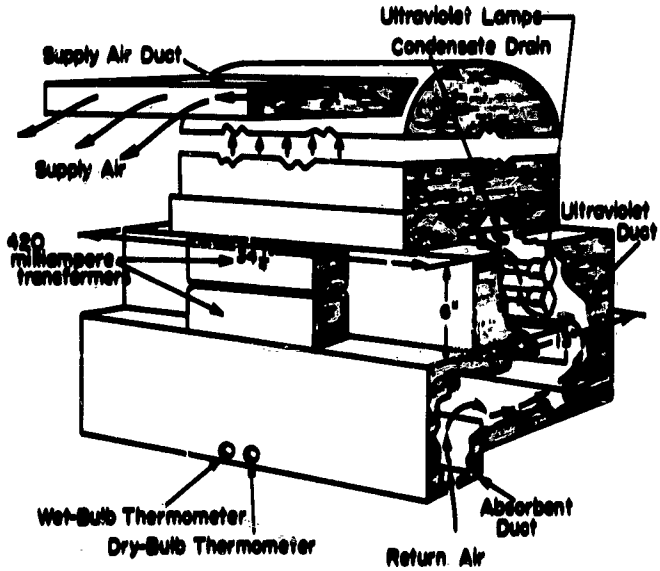


Figure 71. UV Air Conditioner.

The procedure was identical for all experiments. Before each test series, the air conditioner was placed in operation and sufficient time allowed to cool the room air. The intensity of 2537A radiation was measured for each UV lamp as previously described. The UV lamps were turned on, and a control test was made before atomization. A 24-hour broth culture of S. indica diluted in water was then atomized continuously into the test room by a Chicago-type atomizer. Air sampling was initiated after the atomizer had been in operation for a minimum of 30 minutes. The propeller type room fan shown in Figure 70 assisted in mixing the air in the room.

Each test included (a) air samples collected by two sieve samplers located in the supply duct, (b) air samples collected by two liquid impingers placed in the UV-absorbent duct, (c) culture samples of the condensate liquid, and (d) relative humidity and temperature measurements of the air returning to the air conditioner. Each test series consisted of a minimum of five tests.

b. Results

Table LX shows the results of two series of control tests when ultraviolet lamps were not in use, and ten series of tests when the air conditioner was equipped with (a) a single Slimline lamp and (b) two Slimline lamps.

Test series 1 and 2 (Table LX) show the results of control tests when UV radiation was absent. The amounts of the bacterial aerosol which passed through the air conditioner in the absence of UV lamps were 34.9 per cent and 45.0 per cent, respectively. Test organisms were recovered in the condensate in test series 1.

When one Slimline lamp was used, penetrations in the five series of tests (3 through 7) varied from 0.0426 per cent (series 5) to 0.0721 per cent (series 6). The five series of tests were conducted on five different days but under essentially the same conditions. The intensity of the Slimline lamp ranged from 156 to 160 microwatts per square centimeter at one meter. The average penetration of all test series was 0.0535 per cent. No test organisms were recovered in the condensate.

Table LX also shows the results of five series of tests (8 through 12) when two lamps were installed in the system. The penetration varied from 0.0116 per cent in test series 11 to 0.0314 per cent in test series 12. The average penetration was 0.0208 per cent. The combined intensity of the two Slimline lamps ranged from 314 to 326 microwatts per square centimeter at one meter. Test organisms were collected in the condensate in test series 9, 10, and 12.

TABLE LX. USE OF UV IRRADIATION IN A ROOM AIR CONDITIONER FOR REMOVAL OF BACTERIA

TEST SERIES	PER CENT PENETRATION OF TEST ORGANISMS (S. INDICA) THROUGH THE AIR CONDITIONER	AVERAGE PER CENT PENETRATION	RECOVERY OF S. INDICA IN CONDENSATE
	No UV Lamps[a]		
1	35	40	+
2	45		-
	Single Slimline UV Lamp[b]		
3	0.0534		-
4	0.0559	0.0535	-
5	0.0426		-
6	0.0721		-
7	0.0437		-
	Two Slimline UV Lamps[c]		
8	0.0130		-
9	0.0237		+
10	0.0241	0.0208	+
11	0.0116		-
12	0.0314		+

a. Control tests (penetration through air conditioner using no UV lamps).
b. Intensity range from 155 to 160 microwatts per sq cm at one meter.
c. Intensity range from 314 to 326 microwatts per sq cm at one meter.

Test Conditions:
Velocity of air across UV lamps - 200 ft per min
Return air - 280 cfm
Fresh air - 30 cfm
Supply air - 310 cfm
Relative humidity variation - 42 to 51 per cent
Conditioner return air temperature variation - 72.9° to 82.4°F

c. Conclusions

These results show that a 3/4 HP recirculating air conditioner equipped with UV lamps operated at 420 milliamperes substantially reduced the number of air-borne microorganisms in room air by a factor of 99.98 per cent. This reduction was due almost entirely to the germicidal action of UV radiation.

When equipped with UV lamps, the room air conditioner recirculates relatively clean air at constant temperatures and relative humidities and provides the room with purified air comparable to a system which utilizes 100 per cent fresh air. This system also is much more economical than one which requires complete make-up air.

3. Sterilization of Air Flows of One to Ten Cubic Feet Per Minute

Exhaust air from forcibly-aerated bacterial cultures often contain viable organisms (Table LXI). Air from small air-tight chambers used to study the characteristics of bacterial aerosols or used for respiratory challenge of small animals likewise contains residual organisms. In both instances when the organisms are infectious for man, the exhaust air should be sterilized.

TABLE LXI. RECOVERY OF ORGANISMS FROM EXHAUST AIR FROM FORCIBLY AERATED BROTH CULTURES OF S. INDICA[a]

CULTURE	RATE OF AIR FLOW IN CU FT PER MINUTE	DURATION OF SAMPLING, minutes	COLONIES[b] RECOVERED PER LIQUID IMPINGER
50 ml in 250 cc Erlenmeyer flask	0.46	5	4.0×10^4
	0.46	5	4.2×10^4
100 ml in 500 cc Erlenmeyer flask	0.46	5	5.4×10^4
	0.46	5	4.6×10^4
200 ml in 1000 cc Erlenmeyer flask	0.46	5	4.0×10^4
	0.46	5	3.6×10^4

a. 20 cc tryptose broth per impinger.
b. The concentration of the original S. indica cultures was approximately 3.3×10^8 organisms per milliliter.

Possible methods for treatment of such air are electrostatic precipitators, filtration, incineration, and UV irradiation. Although each method has certain advantages and disadvantages, sterilization by UV seems most practical. UV treatment of moving air is not usually considered capable of yielding sterile air because, when large volumes of air are involved, the theoretical number of lamps required becomes so great that physical location is often impractical. For small volumes of air under relatively low flow rates, however, it would appear that a high degree of disinfection may be obtained if the proper amount of energy is employed for the proper time period. UV irradiation offers advantages as follows:

(a) No resistance to air flow,
(b) no significant generation of heat,
(c) relatively inexpensive,
(d) low operational cost,
(e) suitable for small volumes of air,
(f) light weight, and
(g) easily moved from place to place.

Several UV sterilizers for small volumes of air were constructed and tested prior to the development of the apparatus described here. These consisted of one or two high intensity-UV lamps, sometimes in combination with high ozone producing lamps, inclosed in glass or aluminum tubes. Tests showed that these models did not completely sterilize air containing bacterial spores, although all vegetative bacteria were killed. The apparatus described below sterilizes air at a flow of one cubic foot per minute when the air contains as much as 1×10^7 bacterial spores per cubic foot of air.

a. Description of the UV Air Steriliser

The steriliser consists of four aluminum tubes joined alternately top to bottom to provide a continuous flow path for air (Figure 72). Each tube is 35 inches long and 1-3/8 inches in diameter and contains one G36T6 UV lamp. The lamps are held in the tubes by means of rubber stoppers sealed in each end and are operated from an outside ballast supplying 420 milliamperes current to each lamp.

The apparatus has a total volume of approximately 0.092 cubic foot and weighs 20 pounds. It is designed for a flow rate of one cubic foot per minute and a linear flow of approximately two feet per second. At this flow the exposure time of air-borne particles is 5.52 seconds (1.38 seconds per tube). Each UV lamp used produced an intensity of approximately 116 microwatts per square centimeter on a flat surface one meter from the bare lamp and a total UV output of approximately 13 watts. In operation the temperature of the air in the steriliser is 116.6°F.

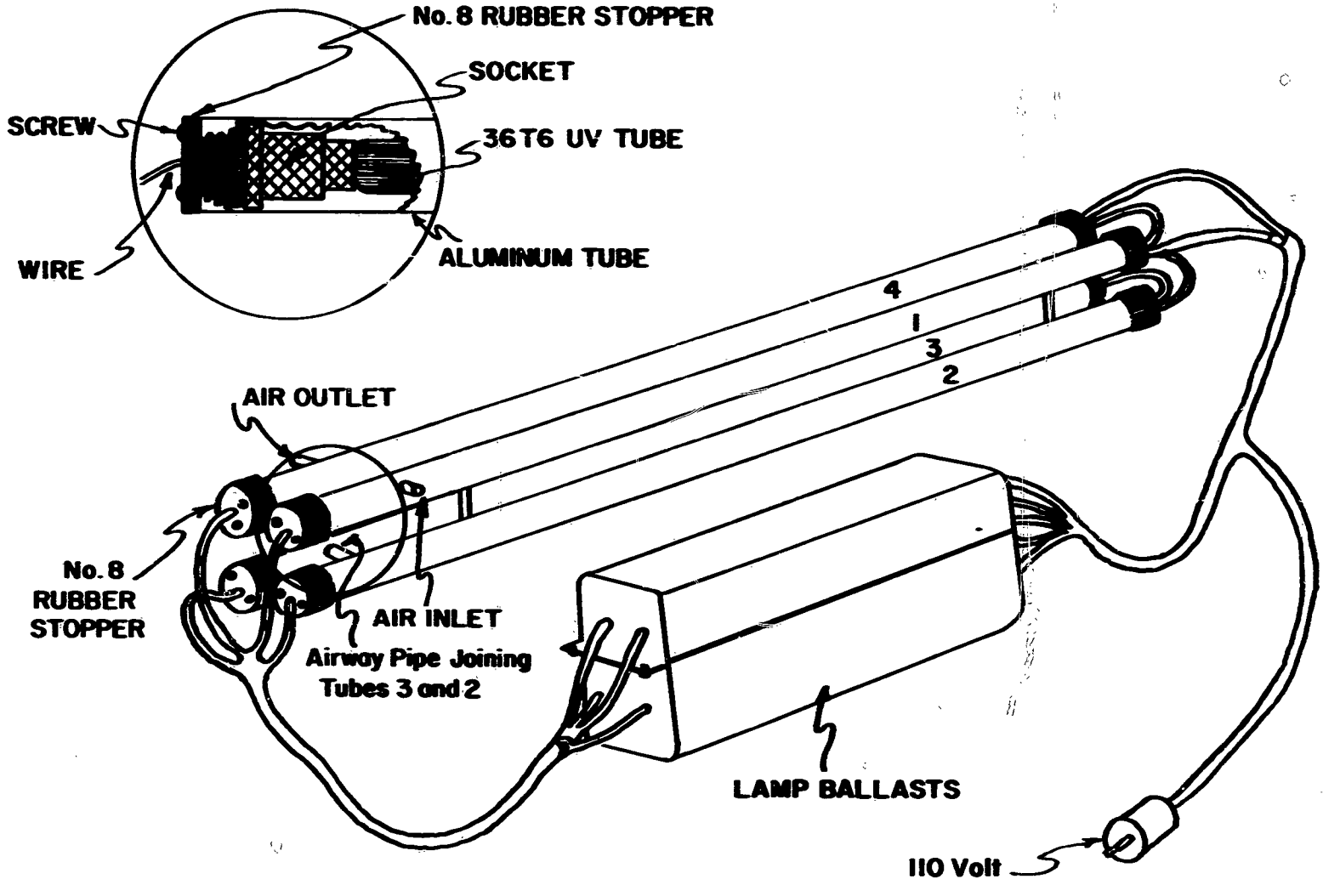


Figure 72. Small Volume UV Air Sterilizer.

b. Test Methods

Spores of Bacillus subtilis var. niger were used as the major test organism. Bacterial aerosols were generated with a Chicago-type glass nebulizer (260) and passed through the steriliser. The exhaust air from the apparatus was sampled with the lamps on and off. The off samples were collected by passing the air through a small tube filled with sterile cotton. Collected spores were subsequently washed from the cotton and plated in the usual manner. The on samples were collected by sieve samplers or slit samplers (58). The concentration of spores entering the tube was determined with cotton samplers.

Suspensions of approximately 1×10^8 spores per millilliter were nebulized at the rate of two to three milliliters per minute for two to four hours continuously. Tests were conducted using one, two, three, and four lamps.

c. Results

Three separate experiments, representing over eight hours of nebulizing, in which the aerosol concentration entering the steriliser varied from 9×10^8 to 5×10^6 spores per cubic foot showed that the use of four lamps inactivated all test organisms in the exhaust air (Table LXII). Experiments in which fewer than four lamps were lighted in the apparatus showed that complete sterility could also be obtained with three lamps, while two lamps and one lamp gave penetrations of 0.000002 and 0.0002 per cent, respectively. Additional tests at increased air flows of four and ten cubic feet per minute showed that aerosols of Serratia indica could be inactivated by four lamps (Table LXII).

The results of these studies show that the apparatus is an efficient means of sterilizing small volumes of highly contaminated air and that the use of four lamps provides a generous safety factor. In practical applications several important maintenance considerations must be kept in mind. Since visual inspection of a lighted UV lamp is not an accurate method of estimating UV output, no inspection windows were provided in the apparatus. The outside temperature of each of the aluminum tubes will indicate whether the enclosed lamp is burning. Records should be kept of the periods of use of the apparatus so that replacement lamps may be installed after approximately 750 hours of use. In addition, the lamps should be cleaned with alcohol after each 1000 hours of use. Small intensity meters, such as the Westinghouse SM-600 meter, may be used periodically to check the UV output. A lamp should be replaced when it tests less than 60 per cent of its initial output (100-hour rating). Formaldehyde vapors may be used to sterilize the entire apparatus before removing the lamps.

TABLE LXII. COMPARATIVE EFFICIENCY OF ONE TO FOUR UV LAMPS IN THE ULTRAVIOLET STERILIZER IN THE PRESENCE OF AEROSOLS OF B. SUBTILIS AND S. INDICA[a]

NUMBER OF RADIANT LAMPS BURNING	TEST ORGANISM	CUBIC FEET OF AIR PER MINUTE	SAMPLING TIME, minutes	ORGANISMS PER CUBIC FOOT ENTERING APPARATUS	ORGANISMS RECOVERED PER CUBIC FOOT	PER CENT PENETRATION
4	B. subtilis spores	1	77	4×10^7	0	0
3	B. subtilis spores	1	10	4×10^7	0	0
2	B. subtilis spores	1	20	4×10^7	1	0.000002
1	B. subtilis spores	1	15	4×10^7	76.5	0.0002
4	Serratia indica	4	10	1×10^8	0	0
4	Serratia indica	10	180	2×10^8	0	0

a. Results are an average of four experiments.

XII. MAINTENANCE OF UV INSTALLATIONS

A. TESTING

Ultraviolet lamps sometimes continue to burn and emit a blue light even after the 2637A output has decreased beyond usefulness. This means that visual inspection of the lamps cannot be employed to judge UV output. Special UV intensity meters must be used. The most useful life of an UV lamp is usually during the time when the lamp is generating between 100 and 60 per cent of its rated UV output. Therefore, lamps are generally discarded when meter readings show that the UV output has fallen below 60 per cent of the 100-hour value.

Routine testing of all UV lamps is an essential part of installation maintenance. Lamps should be tested at intervals which are determined by the type of lamp, the number of hours per month the lamp is in operation, and the frequency of starts. Normally, lamps should be tested every three months except in special locations such as animal rooms and other installations where hot cathode lamps are operated continuously. In general, it is advisable to test all hot cathode lamps more frequently than the cold cathode because of their shorter life expectancy.

The Westinghouse SM-600 meter is preferred for routine lamp testing. Before testing each lamp must be cleaned and allowed to warm up for five minutes. It is common practice to express the lamp intensity readings in terms of microwatts per square centimeter at a distance of one meter from the lamp. The intensity which represents 100 per cent output for each type of lamp is known. An intensity reading of 40 per cent below this figure indicates that the lamp should be replaced. The use of a mimeographed form, Figure 73, is recommended for keeping records of the periodic intensity checks.

B. CLEANING

Wave length 2537A is not particularly penetrating. Dried films from tap water or from disinfectant solutions, grease, oil, or dust on an UV lamp will seriously reduce its output. All lamps should be cleaned routinely at two-week intervals, or more often if the lamps are located in an area which is abnormally dusty. Lamps and reflectors should be wiped with a soft cloth pad which has been moistened with alcohol without being removed from the fixtures. Lamps must be turned off while cleaning.

BLDG.		INSTAL. NO.			CC H15 H30	ROOM		
TYPE		REFLECTOR			EST. USE/DAY			
BULB	DATE	MW	DATE	MW	DATE	MW	DATE	MW

Figure 73. UV Installation Record.

C. DISPOSAL

UV lamps contain mercury vapor and small quantities of metallic mercury, therefore, methods for disposal of used and worn-out lamps should meet the following requirements:

(a) Lamps should be broken in the open so that the mercury vapor will be quickly dissipated.

(b) Liquid mercury from the lamps should not be allowed to enter the building sewer system.

(c) The same care and procedures should be used in the disposal of UV lamps as are used in the disposal of standard fluorescent lamps.

XIII. EFFECTS OF UV RADIATION ON EXPOSED PERSONNEL

A. EFFECTS ON THE EYES AND SKIN

Numerous reports are available on the beneficial and detrimental effects of UV radiation on the body (74,175,181). The effect the radiation will produce is determined by such factors as the dosage of radiation, wave length, the portion of the body exposed, and sensitivity of the individual at the time of irradiation. In the application of ultraviolet lamps, there is always a danger of an accidental over-exposure of the eyes and skin.

1. Effects on the Eyes (Blepharoconjunctivitis)

An over-exposure of the eyes to UV radiation will result in a painful irritation of the conjunctiva and eyelids. The latent period is from three to twelve hours depending upon the amount of radiation received; the greater the exposure, the sooner and more severe are the symptoms. There is a very unpleasant foreign body sensation accompanied by lacrimation. The symptoms usually disappear in a day or two.

a. Medical Effects

Ultraviolet radiation on the eye is absorbed successively by the cornea, the aqueous humor, the lens, and the vitreous humor, before reaching the retina. The relative absorption in these various parts differs. It is greatest in the lens, next in the cornea, then in the vitreous humor, and least in the aqueous humor. With an increase in age there is also an increase of absorption by the lens. The rods and cones are quite sensitive to UV radiation, as has been observed by those who have had a lens removed. About the only effect of UV radiation on the retina that the normal eye can detect is an indirect one; UV radiation of wave length 3600A causes the eye media to fluoresce. The fluorescence produced by the stimulating UV radiation is in the visible spectrum; thus stimulation of the retina results (174).

Wave lengths of less than 2800A are more effective in producing conjunctivitis of the eye than erythema of the skin. This is probably due to the absence of the strongly absorbing horny layer on the conjunctiva. Experiments indicate that the wave lengths causing conjunctivitis are similar to the absorption curve for nucleic acid (174). The characteristic effects on the human eye produced by prolonged exposure to artificial UV sources are: inflammation of the conjunctiva, cornea, and iris; photophobia; copious lacrimation. The cornea is hyperemic, swollen, and covered with a slimy secretion (181).

b. Effects on Mice

Several experiments have been reported pertaining to the effects of UV radiation on the eyes of mice. Buschke et al (40) carried out a series of experiments observing histological as well as microscopic effects. Using the corneal epithelium as the basis for the investigation, they found that mitotic activity of the cells was inhibited. Severe exposures led to nuclear fragmentation in the superficial layers which eventually resulted in the death of the cells. Severe fragmentation is correlated with the clinically visible roughing and stippling of the corneal surface in photophthalmia. These changes are first observed two to three hours after exposure. A loss of cohesion between the epithelium and the stroma occurred with a sloughing off of the loosened cells and, in some cases, layers of cells, in 6 to 48 hours after treatment.

c. Effects on Guinea Pigs

An experiment was carried out in the authors' laboratories to determine the gross effects of UV radiation on forty-two guinea pigs. The pigs were placed one meter from an UV lamp emiting 45 microwatts per square centimeter of 2537A radiation at the distance of one meter. The time of exposure was varied so as to determine the maximum amount of UV radiation that could be tolerated by a guinea pig.

The first exposure was 10 minutes; then 15, 30, and 60 minutes. Thirty-six animals received only a single UV exposure. Four animals were exposed in a series, four times 24 hours apart, and the other two animals were exposed 40 times over a two month period, the minimum interval being 24 hours. Following exposure the eyes and ears of each animal were carefully examined at appropriate intervals for a sustained period of time. An opthalmoscope was employed as necessary.

No significant effects were observed. Guinea pigs appear to suffer no immediate effects from radiation doses of 2537A which are more than enough to cause blepharoconjunctivitis in the human eye. Furthermore, observations for as long as ten months showed no obvious loss of sight or other visual abnormalities.

d. Effects on Humans

The degree of ophthalmia is dependent upon the wave length of radiation as well as the dose. Short wave length radiation, such as 2537A, emitted by bactericidal lamps is mainly absorbed by the conjunctiva and cornea. Some radiation above 3000A is absorbed by the iris and lens as well as the conjunctiva and cornea. The fact that nearly all of the bactericidal (2537A) radiation is absorbed by the immediate surface of the eye would explain why this radiation is more harmful, at a given dosage, than longer radiation that can penetrate through a greater volume of tissue.

Thus Fischer et al (84) reported that about four milliwatt-seconds per square centimeter of 2500A radiation will produce a keratitis while as much as 60 milliwatt-seconds per square centimeter was necessary for radiation at 3000A. Rooks (259) found, on self-investigation, that an exposure of three milliwatt-seconds per square centimeter of 2537A was sufficient to cause a slight ophthalmia occurring 12 hours after exposure. It is, therefore, not surprising that several authors (45,181,259) have emphasized the detrimental effects which can be caused by over-exposure to 2537A radiation. One reported case, resulted in a temporary partial blindness of the patient for eleven days. In a scan of recent literature no case of permanent blindness due to 2537A UV burns could be found, but there have been many cases of conjunctivitis of the eyes which were quite painful for several days. Kovacs (175) reports the use of ultraviolet radiation in the treatment of eye diseases.

e. Conclusions

An accidental over-exposure of the eye to 2537A UV radiation is a painful experience. Records of permanent damage to the eye by this radiation have not been found. To relieve pain Kovacs (175) suggests the use of infrared radiation applied to the closed eyelids. An ordinary incandescent bulb can be held near the eyelids for 20 to 30 minutes. Another method to relieve pain is to bathe the eyes in warm, sterile boric acid solution using sterile cotton pads. This is followed by a drop of sweet oil into each eye. The irritation produced by germicidal lamps disappears within a day or two; much more quickly than a corresponding degree of irritation produced by longer wave length ultraviolet. There is apparently no permanent injury and no hypersensitivity to sunlight as sometimes results from eye burns produced by high intensity quartz mercury arcs, carbon arcs, or welding arcs.

2. Effects on the Skin (Erythema)

a. General Radiation Effects

Exposure of the skin to radiation between 2400A and 3200A will produce an erythema which develops in one to eight hours depending upon the intensity of radiation, the type of radiation, and the sensitivity of the subject. The erythemal effectiveness of different wave lengths is reproduced in Figure 74 from the work of Coblents et al (47).

The effects of UV radiation on the skin have been studied more extensively than effects on the eye. Most experimental work has been done on the formation of erythema. The primary effect is a reddening of the skin, which is due to a temporary increase of blood in the small vessels of the skin.

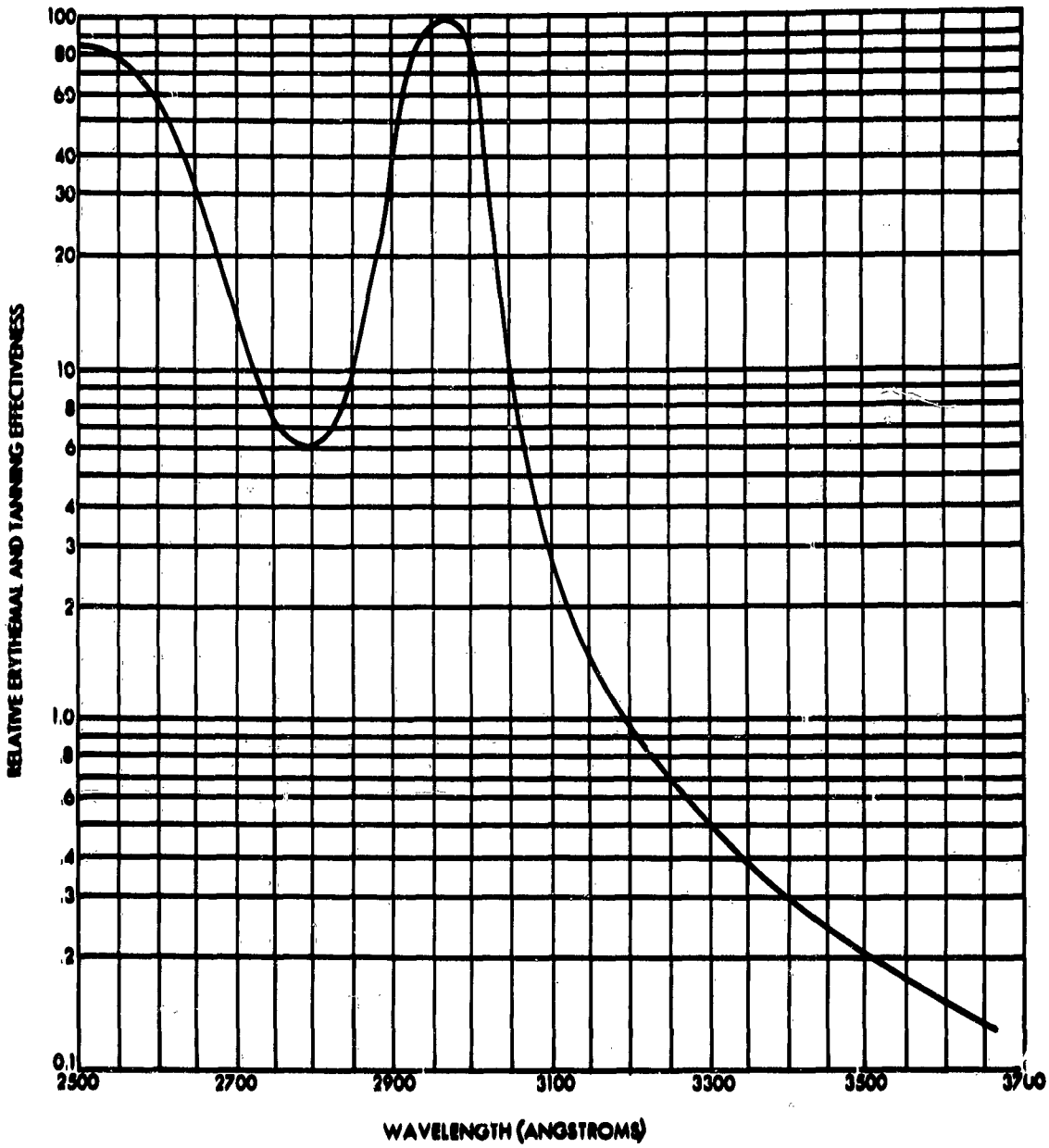


Figure 74. Erythemal Response Curve.

A common biological unit of ultraviolet dosage is the minimum perceptible erythema (MPE) which is equal to approximately 20,000 microwatt-seconds per square centimeter of 2967A radiation equivalent. A MPE disappears in 24 hours. Greater exposure results in various degrees of inflammation or even blistering and hemorrhage. Below is a chart of the degrees of erythema produced by exposure to mid-summer noon-day sun:

Relative Exposure	Degree of Erythema
1	MPE
2.5	Vivid, with moderate tan
5	Painful "burn"
10	Blistering

Erythema is followed by pigmentation (or tanning) of the skin, which is noticeable two to three days after irradiation. Tanning usually does not occur when the erythema is due to 2537A radiation.

Radiation between 2800A and 3200A can cause many reactions in the skin. Some of the radiation can penetrate down through the prickle cells down to the basal cells. About 20,000 microwatt - seconds per square centimeter of radiation at 2967A, the peak of the erythemal curve, will produce a minimum perceptible erythema. There will be a stimulation of the prickle cells to produce pigment and a thickening of the whole epidermis. Large doses of radiation can cause blistering and metabolic disturbances in the entire body as a result of release of photodecomposition products into the blood stream. Ellinger (74) gives examples of increased sensitivity to radiation as a result of application or ingestion of photosensitizing compounds. Both the bactericidal and the longer radiation can convert some of the sterols to vitamin D. Kovacs (175) gives other therapeutic uses of radiation.

The erythemal curve (Figure 74) shows the relative effectiveness of equal amounts of energy in different parts of the spectrum in producing erythema. Thus an exposure of several times the threshold value of solar radiation will produce more severe burns than a similar over-exposure to a germicidal lamp.

Table LXIII summarizes information concerning the transmission of UV through the different layers of the human skin.

Tanning or pigmentation is due to the migration of the pigment already present in the basal cells to the more superficial layers and to the formation of new pigment. The tanning response curve follows the erythema curve in a general way. Migration of the pigment from the undamaged basal cells into the injured cells of the outer epithelial layer is due to the tropic action of the chemical substance set free by the injured cells (181).

TABLE LXIII. PER CENT TRANSMISSION OF UV THROUGH SKIN*

WAVE LENGTH, A	LAYERS OF SKIN			
	Corneum	Malpighi	Corium	Subcutaneous
2000	0	0	0	0
2500	19	11	0	0
2800	15	9	0	0
3000	34	16	0	0
4000	80	57	1	0
5500	87	77	5	0
7500	78	65	21	1
10,000	71	65	17	0
14,000	44	28	9	0

Notes:

2000A - All UV absorbed by the corneum. No radiation reaches the germinatium. Surface organisms are killed. Surface layer of cells also may be destroyed.

2500A to 3000A - Greatest absorption is in the stratum corneum. Some radiation reaches the corium, but none reaches the subcutaneous layers. Erythema and pigment are produced.

3000A to 4000A - Relatively large absorption in the stratum malpighi. There is pigment and tanning produced, but very little erythema.

4000A to 7500A - There is a minimum amount of absorption in the stratum corneum, most of the radiation is absorbed in the corium. Pronounced radiation reaches the subcutaneous layers. Hyperemia is caused.

7500A to 14,000A - There is increased absorption in upper layers, decreasing in lower areas.

* Adapted from Bachem and Reed in Koller (174).

One process which occurs in the skin (the conversion of certain sterols into the vitamins of the D groups) is known to account for the beneficial results of irradiation in the prevention and treatment of rickets.

UV irradiation forms or liberates active substances which are responsible for the erythemal response and tanning. The active substance is probably a protein, or a simple derivative of the cells of the stratum malpighi (germinativum), an H-substance, a substance with some of the functions of histamine, if not histamine itself. It is in the form of a H colloid. It is believed that the injury is caused by the denaturation and coagulation of the proteins of the cells. Protein denaturation by UV radiation has been considered to be a fundamental effect which may lie at the bottom of more complex radiation changes (181).

Repeated irritation by UV rays between 2800A and 3200A (9) can cause chronic lesions which may be precancerous. Malignancy of human skin may result from excessive exposure, perhaps by increasing an already present predisposition, causing a tumor to appear earlier and to become malignant. Erythema and pigmentation are both due to injury to the prickle cell layer of the epidermis, and the production of cancer may be the result of similar photochemical changes.

If the cells of the basal layer of the skin receive an excessive quantity of radiant energy, the two protective processes, cornification and pigmentation, become abnormally great, and a third degenerative process starts. The developing neoplasm occurs in the place of greatest proliferation, beginning in a wart-like hyperkerotosis (cornification, a precancerous change). A cancer develops from a precancerous lesion not only as a result of a continuation of the initial insult but also as a result of any continued trauma. Thus, UV radiation is thought only to play a role in the initiation of the process (181).

b. Effects on Mice

Rusch et al (262) found that UV radiation appears to cause harmful effects in mice quite independent of the processes that lead to tumor formation. With very high intensities of radiation the animals lost weight, their physical condition was visibly poorer, they were less animated, and reproduction ceased.

These workers produced cancerous tumors in test mice, but the time required was two and one-half months. No matter how great the daily dosage, this time could not be reduced. Neither the intensity of the energy nor the length of the daily exposure altered the rate of tumor production.

In the precancerous period there were two phases: (a) the period of exposure, and (b) the latent period. In general the length of the latent period was inversely proportional to the length of the period of exposure.

The existence of a latent period in the development of human cancer is well known, particularly in cancers due to radium, roentgen, and UV radiation. The carcinogenic wave lengths were found to lie between 2900A and 3341A. Wave lengths below 2537A and above 3341A were found to be noncarcinogenic. The carcinogenic wave lengths thus coincided in part with those most potent in the production of erythema. Wave lengths of 2537A produced erythema without producing tumors.

Given over a three-month period, 63 to 84 x 10^7 ergs per square centimeter of effective radiation were adequate to form tumors. Once initiated, the carcinogenic process proceeded without further exposure, as was shown by suspending exposure on one group of animals each day over a period of several weeks. In some cases several months lapsed between the end of the exposure period and the time the tumors appeared.

Blum (29) and Blum et al (31) studied the relationship between dose and rate of tumor induction by UV radiation. The time in which 50 per cent tumor incidence (T50) occurs will not be reduced if the weekly dosage is increased to five days a week. However, increasing the exposure to seven days a week will decrease the induction time. If the weekly dose is given in one exposure, T50 is longer than for five or seven days-a-week exposure. Intensity may be varied over a wide range with no significant change in T50. At high dosages a considerable number of cells are destroyed, thus affecting the induction time because it decreases the amount of tissue in which the tumor may develop.

Blum et al (30) in another study induced 100 per cent incidence of tumors of the ears of mice by exposure to mercury arc radiation under carefully controlled conditions. The production of tumors depends upon the quantity of radiant energy applied rather than upon the intensity of the radiation. There was a wide spread in the time of appearance of the first tumors and of the time of appearance of the last tumors in each series, e.g., 102 days for the first tumor and 221 days for the last tumor. Fifty per cent incidence was reached in 135 days with an exposure dose of approximately 100 MPE's per day.

Photorecovery from the effects of UV radiation was demonstrated by Rieck and Carlson (250) in 1955. Their work was probably the first example of photoreactivation in mammals. Mice were exposed for 35 to 40 minutes a day, 5 days a week for 45 days. The wave lengths of UV radiation were 2000A to 3130A. The highest dose used was 1.6 x 10^8 ergs per square centimeter of radiant energy. Criteria for the detection of effects were: (a) the difference in the death rate of animals kept in darkness as compared to animals exposed to visible illumination between each exposure, and (b) the damage done to the ears of the animals. The results showed that there was about a 35 per cent difference in the death rate. Thirty per cent of the mice kept in the dark survived and about 65 per cent of the mice kept in the light survived. The ears of the animals kept in the dark received much more damage than the ones kept in the light.

c. Effects of 2537A

Radiation from a bactericidal lamp, mainly 2537A, is absorbed by the horny layer and outermost cells of the malpighian layer and never reaches the basal cell layer. The horny layer is the protecting screen for the living epidermal cells. Approximately 30,000 microwatt-seconds per square centimeter of 2537A radiation will produce a minimal perceptible erythema. Greater dosages of 2537A radiation will increase the erythema, followed by the loss of most of the outer layer of the skin. Blistering and hemorrhage of the skin does not occur. Very little or no pigmentation is observed. Laurens (181) states, based on the work of Rusch et al (262), that "radiant energy of 2537A produces erythema but no tumors no matter how large the dose."

The Council of Physical Medicine of the American Medical Association (6) has established a maximum allowable level for 2537A radiation. For persons exposed seven hours daily, the UV intensity falling on the face and hands is limited to 0.5 microwatts per square centimeter of 2537A radiation or a total daily dose of 12,600 microwatt-seconds per square centimeter. This is well under the exposure necessary to produce an MPE on an average untanned exposed skin. The established maximum allowable radiation level for constant exposure has been set at 0.1 microwatt per square centimeter or a total daily dose of 8640 microwatt-seconds per square centimeter. Table LXIV, taken from Buttolph* (151), gives extrapolated values of permissible exposures for different intensities and times.

TABLE LXIV. PERMISSIBLE DAILY EXPOSURE TO UV RADIATION[a]

EXPOSURE TIME PER 24-HOUR PERIOD	INTENSITY OF FACE LEVEL, microwatts per sq cm	TOTAL CALCULATED DOSE, microwatt-minutes per sq cm
24 hours	0.1	144
12 hours	0.3	216
7 hours	0.5	210
6 hours	0.6	216
4 hours	0.9	216
3 hours	1.2	216
2 hours	1.8	216
1 hour	3.6	216
30 minutes	7.2	216
10 minutes	21.6	216
1 minute	216.0	216
5 seconds	2600.0	216

a. Based on American Medical Association standards.

* Cited in Hollaender.

Tests were made by the authors on the white, untanned skin of the upper arm of an individual. An erythemal exposure slide was constructed of cardboard. This slide, when taped to the arm, allowed the intermediate exposure of six circular areas of skin approximately two centimeters in diameter. One 15-watt, hot cathode UV lamp was used as the energy source. The arm of the test individual was held three inches from the center of the lamp. At this point the intensity falling upon the skin was 1000 microwatts per square centimeter. Six circular areas of skin were exposed to the UV from 0.5 to 8 minutes. The ET values received varied from 500 in the 0.5 minute exposure to 8000 in the 8 minute exposure. After exposure, the skin was observed for a period of 24 hours. For this test individual the following results were observed:

(a) Skin areas receiving an ET of 500 showed a (MPE) minimum perceptible erythema (very light pink).

(b) Skin areas receiving an ET of 1000 to 3000 showed definite erythema with increasingly darker pink discoloration of the skin. The areas were not painful to the touch.

(c) Skin areas receiving an ET of 8000 had moderate to severe erythema. The areas were medium to dark red in color and were slightly sensitive to the touch. No blister formation resulted.

The ET value required to produce a MPE on this individual was about the same as the value given by Luckiesh.

An experiment was conducted with six adult males to determine if any injurious effects could be caused by short exposures to UV radiation in an air lock. The air lock used was eight feet long, three feet six inches wide, and ten feet high. The radiation was supplied by three bare 30-watt, hot cathode lamps mounted in the ceiling. The average UV intensity in the air lock was 79 microwatts per square centimeter at a five-foot level above the floor and 129 microwatts per square centimeter at the seven-foot level.

The six individuals were exposed to the radiations in the air lock for periods of time varying from 10 to 60 seconds. Three of the individuals wore their personal eye glasses, three did not. All were bareheaded. Five of the men had bare arms and shoulders during the test which was conducted during the winter months when the exposed skin areas were white and untanned. None of the exposed individuals experienced any degree of eye burn (conjunctivitis) and no perceptible erythema was noted on the skin. It was estimated that the maximum time that would be required for an individual to pass through this eight-foot air lock under normal circumstances was five seconds. The 60-second exposure did not affect the eyes or skin of the men. Therefore, normal passage through the air lock when the UV lamps were operating was considered to be safe.

d. Conclusions

When evaluating the hazards of UV radiation, the effect on the eyes should be given first attention. If the intensities involved are within the allowable limits set by the American Medical Association, no protection for the eyes or skin is required. Slightly higher intensities may require protection for the eyes, and when very high intensities are present, it may be necessary to protect the skin as well as the eyes. Justification for the protection of the eyes before the skin stems from the fact that the eyes are more sensitive and that no real harm results from a minimum erythema of the skin; the skin usually adapts itself rather rapidly.

B. PERSONNEL PROTECTION

The general problem of protection of personnel from injury by UV radiation may be divided into two categories. Under the first category such visual aids as warning signs and indicator lights may be considered, while the second includes protective equipment to be worn by exposed personnel.

Some specific rules for the use of visual aids are listed below:

(a) When UV lamps are controlled by manual switches, the switches should be located outside the room, preferably near the entrance door.

(b) When manual switches are used, a small colbalt blue indicator light should be mounted near the switch. The indicator light will serve as a constant reminder that the UV lamps are burning.

(c) Warning signs must be used at every UV installation. The exact location of the signs and the message they convey will vary with different types of installations. In general it is desirable to post a sign outside a room housing an UV installation. The wording on the sign will coincide with the safety regulations recommended for the particular type of installation. The sentences listed below illustrate the type of message to be used on the signs:

(1) Caution - Ultraviolet lamps in use, protect your eyes.

(2) Caution - Ultraviolet lamps in use, do not enter.

(3) Caution - Turn off ultraviolet lamps before entering.

(4) Caution - Strong ultraviolet in use, protect your skin and eyes.

(d) In some installations (such as UV door barriers) a danger pattern may be painted on the floor or walls to designate areas of high UV intensity.

UV radiation in the 2537A range has little penetrating effect. Ordinary glass completely absorbs the energy, as do most plastics, rubber, and similar materials. The penetration of UV through clothing will depend upon the closeness of weave of the fabric. Practical experience has shown that the skin is usually adequately protected by ordinary cotton laboratory clothing.

1. Eye Protection

While ordinary spectacles will in many instances offer adequate eye protection, it is recommended that safety glasses or goggles with solid side pieces be used. The side pieces prevent the entrance of the radiation when the source is to the left or right of the exposed individual. Cases of eye conjunctivitis have been known to occur when the individual wore ordinary spectacles.

2. Skin Protection

Installations requiring skin protection also require eye protection. The main portion of the body and the arms, and legs are protected by ordinary clothing. Rubber or cotton gloves can be used to protect the hands. A plastic personnel hood (Figure 75) may be conveniently used to protect the eye, head, and neck. In some cases face shields adequately protect the face and eyes. If the face shield is used, it is recommended that some type of cap be worn to protect the area of the upper part of the head. Personnel working in areas where respirators are required can be provided with a modified face shield as illustrated in Figure 75. The type of equipment used when UV radiation is installed in hospital operating rooms is illustrated in Figure 32.

In installations where personnel are exposed to high intensities for long periods of time, it has been necessary to wear safety goggles in addition to plastic personnel hoods (e.g., animal rooms with UV cage racks). This is because of penetration of the plastic by the longer UV wave lengths emitted in small quantities by the low pressure mercury vapor lamps.

Whenever plastic items, such as face shields, are used for UV radiation protection, tests should be made to assure that the formulation has zero transmission of 2537A. Lucite face shields, for example, have on occasion been found to transmit germicidal radiation.

Figure 75. Plastic Personnel Hood for Protection Against UV Radiation. (FD Neg C-3389)

LITERATURE CITED

1. Aerobiology: Publication 17, American Association for the Advancement of Science, Washington, D.C., 1942.

2. Allen, E.G.; Bovarnick, M.R.; and Snyder, J.C.: "The Effect of Irradiation with Ultraviolet Light on Various Properties of Typhus Rickettsiae," J Bact, 67:718-723, 1954.

3. Allen, J.G.: "The Riddle of Homologous Serum Jaundice," Proc Amer Assoc Blood Banks, p. 11, 1951.

4. Allen, J.G.; Sykes, C.; Enerson, D.M.; Moulder, P.V.; Elghammer, R.M.; Grossman, B.J.; McKeen, C.L.; and Galluzzi, N.J.: "Homologous Serum Jaundice and Its Relation to Methods of Plasma Storage," J Amer Med Assoc, 144:1069-1074, 1950.

5. American Medical Association: "Threshold Limit Values for 1958," A.M.A. Arch Ind Health, 18:178-182, 1958.

6. American Medical Association: "Council on Physical Medicine: Acceptance of Ultraviolet Lamps for Disinfecting Purposes," J Amer Med Assoc, 137:1600-1603, 1948.

7. American Medical Association: "Council on Physical Therapy: Acceptance of Sunlamps," J Amer Med Assoc, 114:325-326, 1940.

8. Anderegg, F.O.: "Recent Progress in the Use of Ozone in Ventilation," Proc Indiana Acad Sci, 271-273, 1920.

9. Anderson, T.F.: "Virus Reactions Inside of Bacterial Host Cells," J Bact, 47:113, 1944.

10. Anderson, W.T., Jr.: "Ultraviolet Sanitary Ventilation," Heating and Ventilating, 44:63-68, 1947.

11. Antara Chemicals Bulletin: "Uvinuls, Ultraviolet Light Absorbers," Antara Chemicals, 435 Hudson Street, New York 14, New York, 1956.

12. Applemans, R.: "Quelques applications de la methods de dosage du bacteriophage," Compt Rend Soc Biol, 86:508-509, 1922.

13. Arloing, S.: "Influence de la lumiere sur la vegetation et les proprietes pathogenes du Bacillus anthracis," Compt Rend, 101:378-381, 1885.

14. Arloing, S.: "Influence de soleil sur vegetabilite des spores du Bacillus anthracis," Compt Rend, 101:511-513, 1885.

15. Armour Research Foundation: Personal Communication, 1955.

16. Bahlke, A.M.; Silverman, H.F.; and Ingraham, H.S.: "Effect of Ultraviolet Irradiation of Classrooms on Spread of Mumps and Chickenpox in Large Rural Central Schools," Amer J Public Health, 39:1321-1330, 1949.

17. Bang, S.: "Die Wirkungen des Lichtes Auf Mikro-organismen," Mitt Finsens Med Lysinst, 9:164, 1905.

18. Barger, G.J.P., and Knott, E.K.: "Blood: Ultraviolet Irradiation (Knott Technic)," Med Physics, 2:132-136, 1950.

19. Barnard, J.E., and Morgan, H.: "Upon the Bactericidal Action of Some Ultraviolet Radiations as Produced by the Continuous Current Arc," Proc Roy Soc, London, 72:126-128, 1903.

20. Barnett, R.N.; Fox, R.A.; and Snavely, J.G.: "Hepatitis Following the Use of Irradiated Human Plasma," J Amer Med Assoc, 144:226-228, 1950.

21. Bartell, A.W., and Temple, J.W.: "Ozone in Los Angeles and Surrounding Areas," Ind Eng Chem, 44:857-861, 1952.

22. Bass, F.: "An Experiment with Ozone in School Ventilation," Amer J Public Health, 3:1135-1137, 1913.

23. Bedford, T.H.B.: "The Nature of the Action of Ultraviolet Light on Microorganisms," Brit J Exptl Path, 8:437-441, 1927.

24. Beebe, J.M., and Pirsch, G.W.: "Response of Air-Borne Species of Pasteurella to Artificial Radiation Simulating Sunlight under Different Conditions of Relative Humidity," Appl Microbiol, 6:127-138, 1958.

25. Benedict, F.G.: "The Composition of the Atmosphere with Special Reference to Its Oxygen Content," Carnegie Institute Washington Publ 166, 1912.

26. Benesi, E.: "Design of a Centrifugal Filmer for Ultraviolet Irradiation of Liquids," Gen Motors Eng J, 3:2-8, 1956.

27. Blake, A.F.: "Ray-Table Unit Curbs Bacteria on Sugar," Food Industries, 23:77, 1951.

28. Blanchard, M.C.; Stokes, J.; Hampil, B.; Wade, G.R.; and Spizzizen, J.: "Methods of Protection Against Homologous Serum Hepatitis. II. The Inactivation of Hepatitis Virus SH with Ultraviolet Rays," J Amer Med Assoc, 138:341-343, 1948.

29. Blum, H.F.: "On the Mechanism of Cancer Induction by Ultraviolet Radiation," J Natl Cancer Inst, 11:463-493, 1950.

30. Blum, H.F.; Kirby-Smith, J.S.; and Grady, H.G.: "Quantitative Induction of Tumors in Mice with Ultraviolet Radiation," J Natl Cancer Inst, 2:259-268, 1941.

31. Blum, H.F.; Grady, H.G.; and Kirby-Smith, J.S.: "Relationship Between Dosage and Rates of Tumor Induction by Ultraviolet Radiation," J Natl Cancer Inst, 3:91-97, 1942.

32. Blundell, G.P.; Erf, L.A.; Jones, H.W.; and Hoban, R.T.: "Observations on the Effect of Ultraviolet Irradiation (Knott Technic) on Bacteria and Their Toxins Suspended in Human Blood and Appropriate Diluents," J Bact, 47:85-96, 1944.

33. Bordas, F.: "The Destruction of Bacterial Dust and the Deodorization of Gases," Tech Sanit et Munic, 17:56-60, 1922.

34. Bourdillon, R.B.; Lidwell, O.M.; and Lovelock, J.E.: "Studies in Air Hygiene," Medical Research Council Special Report Series 262, His Majesty's Stationery Office, London, 1948.

35. Boyer, J.: "La desodorisation des tunnels du Metropolitan pas l'ozone," Nature, Paris, 53:2680:97-99, 1925.

36. Bozeman, V.; Tripp, J.T.; and Berry, B.: "A Modified Habel-Sockrider Ultraviolet Irradiation Apparatus for Use in Serum and Vaccine Production," J Immunol, 64:65-72, 1950.

37. Bradley, C.E., and Haagen-Smit, A.J.: "The Application of Rubber in the Determination of Ozone," Rubber Chem and Technol, 24:750-755, 1951.

38. Bucholz, J., and Von Jeney, A.: "Uber das Wesen der bakteriziden Wirkung von monochromatischen ultravioletten Strahlen," Zentr Bakteriol, Parasitenk, Abt. I-orig, 133:5/6:299-304, 1935.

39. Bunker, J.W.M., and Harris, R.S.: "Precise Evaluation of Ultraviolet Therapy in Experimental Rickets," New Engl J Med, 216:165-169, 1937.

40. Buschke, W.; Friedenwald, J.S.; and Moses, S.G.: "Effects of Ultraviolet Radiation on Corneal Epithelium: Mitosis, Nuclear Fragmentation Post Traumatic Cells, Movements Loss of Tissue Cohension," J Cellular Comp Physiol, 26:147-164, 1945.

41. Calmette, A.; Roux, Bouries, Buisine; and Staes-Brame: "Rapport sur la sterilisation industrielle des eaux potables par l'ozone," Ann Inst Pasteur, 13:344-357, 1899.

42. Carlson, H.J.; Ridenour, G.M.; and McKhann, C.F.: "Efficacy of Standard Purification Methods in Removing Poliomyelitis Virus from Water," Amer J Public Health, 32:1256-1262, 1942.

43. Carlson, J.G.; Hollaender, A.; and Gaulden, M.E.: "Ultraviolet Radiation as a Means of Sterilizing Tissue Culture Materials," Science, 105:187-188, 1947.

44. Cernovodeanu, P., and Henri, V.: "Action de la lumiere ultra-violette sur la toxine tetanique," Compt Rend, 149:365-368, 1910.

45. Coblentz, W.W.: "The Hazard of Burns from Artificial Ultraviolet Applications," Arch Phys Therapy, 23:709-711, 1942.

46. Coblentz, W.W., and Fulton, H.R.: "A Radiometric Investigation of the Germicidal Action of Ultraviolet Radiation," Natl Bur Standards (U.S.), Sci Technol Papers 495, 19:641-680, 1924.

47. Coblentz, W.W.; Stair, R.; and Hogue, J.M.: "The spectral Erythemal Reaction of Untanned Skin to Ultraviolet Light," Research Paper 433, Bur Standards J Research, 8:541-547, 1932.

48. Commission on Acute Respiratory Diseases: "A Laboratory Outbreak of Q Fever Caused by the Balkan Grippe Strain of Rickettsia burnetii," Amer J Hyg, 44:123-157, 1946.

49. Cortelyou, J.R.; McWhinnie, M.A.; Riddiford, M.S.; and Semrad, J.E.: "Effects of Ultraviolet Irradiation on Large Populations of Certain Water-Borne Bacteria in Motion. I. The Development of Adequate Agitation to Provide an Effective Exposure Period," Appl Microbiol, 2:227-235, 1954.

50. Crabtree, J.: Paper presented to New Jersey section, American Chemical Society, 1951.

51. Crabtree, J., and Kemp, A.R.: "Weathering of Soft Vulcanized Rubber," Ind Eng Chem, 38:278-296, 1946.

52. Crabtree, J., and Erickson, R.H.: "Atmospheric Ozone-A Simple Approximate Method of Measurement," India Rubber World, 125:719-720, 1952.

53. Cutler, S.S.; Burbank, B.; and Marzullo, E.R.: "Hypocoagulability of Certain Irradiated Plasma," J Amer Med Assoc, 143:1057-1059, 1950.

54. Czaplewski, Prof.: "Uber die Verwendung des Ozons bei der Luftung in hygienischer Beziehung," Gesundh Ingr, 36:565-568, 1913.

55. Czaplewski, Prof.: "Hygiene and the Use of Ozone for Ventilation," Met Chem Eng, 12:254-258, 1913.

56. Dalla Valle, J.M.: Exhaust Hoods: How to Design for Efficient Removal of Dust, Fumes, Vapors and Gases, with Data, Formulas and Practical Examples Showing Exact Procedure, Industrial Press, 148 Lafayette Street, New York 13, New York, 1945.

57. Davidovich, P.: "Commercial Glass Transmitting Ultraviolet Light," J Opt Soc Amer, 20:627-641, 1930.

58. Decker, H.M., and Wilson, M.E.: "A Slit Sampler for Collecting Airborne Microorganisms," Appl Microbiol, 2:267-269, 1954.

59. Decker, H.M.; Geile, F.A.; Harstad, J.B.; and Gross, N.H.: "Spun Glass Air Filters for Bacteriological Cabinets, Animal Cages and Shaking Machine Containers," J Bact, 63:377-383, 1952.

60. Demerec, M., and Latarjet, R.: "Mutations in Bacteria Induced by Radiations," Cold Spring Harbor Symposia Quant Biol, 11:38-49, 1946.

61. Deming, H.G.: Fundamental Chemistry, 2nd Edition, John Wiley and Sons, New York, 1947. pp. 86-87.

62. Dennington, A.R.: "Safety Requirements for Sterilamps in Air Conditioning Systems," Heating and Ventilating, 40:46-49, 1943.

63. Dennis, L.M.: Gas Analysis, Macmillan Company, New York, 1914. p. 192.

64. Dickerman, J.M.; Castraberti, A.O.; and Fuller, J.E.: "Action of Ozone on Waterborne Bacteria," J New Engl Water Works Assoc, 68:11-15, 1954.

65. Dillon-Weston, W.A.R.: "Effect of Ultraviolet Radiation on the Uredospores of Some Physiologic Forms of Puccinia graminis tritici," Sci Agric, 12:81-87, 1931.

66. Dorcas, M.J.: "Ultraviolet in Industry," Trans Illum Eng Soc, New York, 28:575-589, 1933.

67. Downes, J.: "Control of Acute Respiratory Illness by Ultraviolet Lights," Amer J Public Health, 40:1512-1520, 1950.

68. Downs, A., and Blunt, T.P.: "Research on the Effects of Light Upon Bacteria and Other Organisms," Proc Roy Soc, London, 26:488-500, 1878.

69. Duclaux, E.: "Influence de la lumiere du soleil sur la vitalite des germs de microbes," Compt Rend, 100:119-121, 1885.

70. Duggar, B.M.: Effects of Radiation on Bacteria, in Biological Effects of Radiation, B. M. Duggar, Editor, McGraw-Hill Book Company, Inc., New York, 1936. pp. 1119-1149.

71. Duggar, B.M., and Hollaender, A.: "Irradiation of Plant Viruses and of Microorganisms with Monochromatic Light. II. Resistance to Ultraviolet Radiation of a Plant Virus as Contrasted with Vegetative and Spore Stages of Certain Bacteria," J Bact, 27:241-256, 1934.

72. Ehrisman, O., and Noethling, W.: "Uber die bactericide Wirkung monochromatischen Lichtes," Z Hyg Infektionskrankh, 113:597-628, 1932.

73. Elford, W.J., and Van de Ende, J.: "An Investigation of the Merits of Ozone as an Aerial Disinfectant," J Hyg, 42:240-265, 1942.

74. Ellinger, F.: "Ultraviolet Radiation-Its Role in Industrial Medicine," Ind Med, 13:298-300, 1944.

75. Ellis, C.; Wells, A.A.; and Heyroth, F.F.: The Chemical Action of Ultraviolet Rays, 2nd Edition, Reinhold Publishing Corporation, New York, 1941.

76. Erlansden, A., and Schwartz, L.: "Experimentelle Untersuchungen uber Luftozonisierung," Z Hyg Infektionskrankh, 67:391-428, 1910.

77. Ewell, A.W.: "Ozone and Light in Refrigeration," Refrigeration Data Book, Vol. 2, 1940. pp. 199-203.

78. Ewell, A.W.: "Production, Concentration, and Decomposition of Ozone by Ultraviolet Lamps," J Appl Phy, 13:759-767, 1942.

79. Ewell, A.W.: "Refrigeration Limitations and Aids," Refrigeration Eng, 50:523-524, 560-561, 1945.

80. Ewell, A.W.: "Ozone and Light," Refrigeration Data Book, 2nd Edition, Chapter 18, 1946. pp. 193-199.

81. Ewell, A.W.: "Recent Ozone Investigations," J Appl Phy, 17:908-911, 1946.

82. Ewell, A.W.: "The Present Status of Ozone," Ice and Refrig, 120:4:55-56, 1951.

83. Feldner, A.M.: "An Experiment with Ozone as an Adjunct to Artificial Ventilation," Heating and Ventilating, 12:3:35-36, 1915.

84. Fischer, F.P.; Vermeulen, D.; and Eymers, J.: "Uber die zur Schadigung des Auges notige Minimalquantitat von ultravioiettem and infrarotem Licht," Arch Augenheilk, 109:462-467, 1936.

85. Fluke, D.J., and Pollard, E.C.: "Ultraviolet Action Spectrum of T_1 Bacteriophage," Science, 110:274-275, 1949.

86. Foulk, C.V., and Bawden, A.T.: "A New Type of End-Point in Electrometric Titration and Its Application to Iodimetry," J Amer Chem Soc, 48:2045-2051, 1926.

87. Franklin, M.W.: "Air Ozonization," J Ind Eng Chem, 6:850-855, 1914.

88. Franklin, R.M.; Friedman, M.; and Setlow, R.B.: "The Ultraviolet Action Spectrum of a Bacillus megatherium Bacteriophage," Arch Biochem Biophys, 44:259-264, 1953.

89. Fraser, R.: "Ultraviolet Radiation in Surgery," Can Med Assoc J, 51: 403-409, 1944.

90. Friedoriszick, F.K.: "Neue Erfahrungen mit der ultravioletten Luftentkeimung in der Kinderklinik," Strahlentherapie, 95:491-495, 1954.

91. Fulton, H.R., and Coblentz, W.W.: "The Fungicidal Action of Ultraviolet Radiation," J Agric Research, 38:159-168, 1929.

92. Garvie, E.I.: "The growth of Escherichia coli in Buffer Substrate and Distilled Water," J Bact, 69:393-398, 1955.

93. Gates, F.L.: "On Nuclear Derivatives and Lethal Action of Ultraviolet Light," Science, 68:479-480, 1928.

94. Gates. F.L.: "A Study of the Bactericidal Action of Ultraviolet Light," J Gen Physiol, 13:231-260, 1929.

95. Gates, F.L.: "Results of Irradiating Staphylococcus aureus Bacteriophage with Monochromatic Ultraviolet Light," J Exptl Med, 60:179-188, 1934.

96. Gelpherin, A.; Granoff, M.A.; and Linde, J.I.: "The Effect of Ultraviolet Light Upon Absenteeism from Upper Respiratory Infections in New Haven Schools," Amer J Public Health, 41:796-805, 1951.

97. General Electric Company: Bulletin LD-11, General Electric Engineering Division, Lamp Dept, Cleveland 12, Ohio, November 1951.

98. General Electric Company: Bulletin LD-14, General Electric Engineering Division, Lamp Dept, Cleveland 12, Ohio, September 1953.

99. General Electric Company: Lamp Bulletin LD-1, General Electric Engineering Division, Lamp Dept, Cleveland 12, Ohio.

100. Giese, A.C.: "Action of Ultraviolet Radiation on Protoplasm," Physiol Revs, 30:431-458, 1950.

101. Giese, A.C., and Christensen, E.: "Effects of Ozone on Organisms," Physiol Zool, 27:101-115, 1954.

102. Giese, A.C.; Iverson, R.M.; and Sanders, R.T.: "Effect of Nutritional State and Other Conditions on Ultraviolet Resistance and Photoreactivation in Yeast," J Bact, 74:271-279, 1957.

103. Gilcreas, F.W., and Roberts, H.V.: "Bacteriologic Studies in Disinfection of Air in Large Rural Central Schools. I. Ultraviolet Irradiation," Amer J Public Health, 40:808-812, 1950.

104. Gilcreas, F.W., and Read, H.R.: "Bacteriologic Studies in Disinfection of Air in Large Rural Central Schools. II. Ultraviolet Irradiation and Triethylene Glycol Vapor Treatment," Amer J Public Health, 45:767-773, 1955.

105. Gluckauf, E.; Heal, H.G.; Martin, G.R.; and Paneth, F.A.: "A Method for the Continuous Measurement of the Local Concentration of Atmospheric Ozone," J Chem Soc, Part 1, 1-4, 1944.

106. Greene, D.; Barenberg, L.H.; and Greenberg, B.: "Effect of Irradiation of the Air in a Ward on the Incidence of Infections of the Respiratory Tract with a Note on Varicella," Amer J Diseases Children, 55:273-275, 1941.

107. Greene, G.T., and Babel, F.J.: "Effect of Ultraviolet Irradiation on Bacteriophage Active Against Streptococcus lactis," J Dairy Sci, 31: 509-515, 1948.

108. Greenlee, R.G.; Terrill, R.J.; and Sloan, J.Q.: "Homologous Serum Hepatitis After Transfusions of Blood and Ultraviolet Irradiated Plasma," Texas State J Med, 47:831-835, 1951.

109. Guyton, H.G.; Buchanan, L.M.; and Lense, F.T.: "Evaluation of Respiratory Protection of Contagion Masks," Appl Microbiol, 4:141-143, 1956.

110. Habel, K., and Sockrider, B.T.: "A Continuous Flow Method of Exposing Antigens to Ultraviolet Radiation," J Immunol, 56:273-279, 1947.

111. Haines, R.B.: "Effect of Pure Ozone on Bacteria," Report of Food Investigation Board for Year 1934, His Majesty's Stationery Office, London, 1935. pp. 44-46.

112. Haines, R.B.: "The Effect of Pure Ozone on Bacteria," Report of Food Investigation Board for Year 1935, His Majesty's Stationery Office, London, 1936. pp. 30-31.

113. Hall, H.H., and Keane, J.C.: "Effect of Radiant Energy on Thermophilic Organisms in Sugar," Ind Eng Chem, 31:1166-1170, 1939.

114. Hallett, E.S.: "Ozone as the Solution of the Fresh Air Problem," Heating and Ventilating, 17:25-29, 1920.

115. Hann, V.A., and Manley, T.C.: "Ozone," Encyclopedia of Chemical Technology, Vol. 9, 1952. pp. 735-753.

116. Hanovia Chemical Company: Data Sheet 8-49, Hanovia Chemical and Manufacturing Company, 100 Chestnut Street, Newark 5, New Jersey.

117. Hardwick, W.A., and Foster, J.W.: "On the Nature of Sporogenesis in Some Aerobic Bacteria," J Gen Physiol, 35:907-927, 1952.

118. Harstad, J.B.; Decker, H.M.; and Wedum, A.G.: "Use of Ultraviolet Irradiation in a Room Air Conditioner for Removal of Bacteria," Appl Microbiol, 2:148-151, 1954.

119. Hart, D.: Private Communication.

120. Hart, D.: "Sterilization of Air in Operating Rooms by Special Bactericidal Radiant Energy," J Thoracic Surg, 6:45-81, 1936.

121. Hart, D.: "Sterilization of Air in the Operating Room with Bactericidal Radiation," J Thoracic Surg, 7:525-535, 1938.

122. Hart, D.: "Pathogenic Bacteria in the Air of Operating Rooms. Their Widespread Distribution and the Methods of Control," Arch Surg, 37: 521-530, 1938.

123. Hart, D.: "Sterilization of the Air in the Operating Room by Bactericidal Radiant Energy. Results in Over 800 Operations," Arch Surg, 37:956-972, 1938.

124. Hart, D.: "Sterilization of the Air in the Operating Room with Bactericidal Radiation. Results from November 1, 1938 to November 1, 1939, with a Further Report as to Safety of Patients and Personnel," Arch Surg, 41:334-350, 1940.

125. Hart, D.: "The Importance of Airborne Pathogenic Bacteria in the Operating Room: A Method of Control by Sterilization of the Air with Ultraviolet Radiation," J Amer Med Assoc, 117:1610-1613, 1941.

126. Hart, D.: "The Importance of Airborne Pathogenic Bacteria in the Operating Room: A Method of Control by Sterilization of the Air with Ultraviolet Radiation," Aerobiology, Publication 17, AAAS, Washington, D.C., 1942.

127. Hart, D., and Upchurch, S.E.: "Post-Operative Temperature Reactions: Reductions Obtained by Sterilizing the Air with Bactericidal Radiant Energy. Seasonal Variations," Ann Surg, 110:291-306, 1939.

128. Hart, D., and Jones, R., Jr.: "The Prevention of Infection by Airborne Bacteria of Operative Wounds," J Med Assoc, Georgia, 29:401-404, August 1940.

129. Hart, D.; Devine, J.W.; and Martin, D.W.: "Bactericidal and Fungicidal Effect of Ultraviolet Radiation. Use of a Special Unit for Sterilizing the Air in the Operating Room," Arch Surg, 38:806-815, 1939.

130. Hart, D.; Schiebel, H.M.; and Sharp, D.G.: "Disinfection of the Air in the Operating Room with Bactericidal Radiant Energy," Trans South Surg Assoc, 54:347-372, 1942.

131. Hartman, F.E.: "Ozone Generators and the Industrial Application of Ozone," J Soc Chem Ind, 42:117-126, 1923.

132. Hartman, F.W.; LoGrippo, G.S.; and Kelly, A.R.: "Procedures for Sterilization of Plasma Using Combination of Ultraviolet Irradiation and Beta-Propiolactone," Federation Proc, 15:518, 1956.

133. Haynes, H.: Personal Communication, General Electric Corporation, July 1953.

134. Heiling, A., and Scupin, L.: "Action of Ozone in Fruit and Vegetable Cold Rooms," Chem Absts, 31:5401, 1937.

135. Heinmets, F.; Taylor, W.W.; and Lehman, J.J.: "The Use of Metabolites in the Restoration of the Viability of Heat and Chemically Inactivated Escherichia coli," J Bact, 67:5-12, 1954.

136. Heinmets, F.; Lehman, J.J.; Taylor, W.W.; and Kathan, R.H.: "The Study of Factors which Influence Metabolic Reactivation of the Ultraviolet Inactivated Escherichia coli," J Bact, 67:511-522, 1954.

137. Henderson, Y., and Haggard, H.W.: "Noxious Gases and the Principles of Respiration Influencing Their Action," Monograph 35, American Chemical Society, 2nd Edition, Reinhold Publishing Corporation, New York, 1943.

138. Henle, W.; Sommer, H.E.; and Stokes, J.: "Airborne Infection in Hospital Ward: Effects of Irradiation and Propylene Glycol Vaporization Upon Prevention of Experimental Airborne Infection of Mice by Droplet Nuclei," J Pediat, 22:577-590, 1942.

139. Henri, V.: "Etude de l'action metabiotique des rayons ultraviolettes. Production de formes de mutation de la bacteridie charbonneuse," Compt Rend, 158:1032-1035, 1914.

140. Hercik, F.: "Action of Ultraviolet Light on Spores and Vegetative Forms of Bacillus Megatherium sp.," J Gen Physiol, 20:589-594, 1937.

141. Heubner, R.J.: "Report of an Outbreak of Q Fever at National Institutes of Health," Amer J Public Health, 37:431-440, 1947.

142. Hibben, S.G., and Blackburg, P.W.: "Sterilization by Ultraviolet Radiation," Electrical Eng, 57:455-459, 1938.

143. Higgons, R.A., and Hyde, G.M.: "Observations After One Year of Ultraviolet Air Sterilization in a Children's Hospital," Hospitals, 17:75-78, 1943.

144. Higgons, R.A., and Hyde, G.M.: "Effect of Ultraviolet Air Sterilization Upon Incidence of Respiratory Infections in a Children's Institution - A Six Year Study," N.Y. State J Med, 47:707-710, 1947.

145. Hill, E.V.: "Pure Ozone Effect in Air Conditioning," Intern Eng, 82:101-109, 1942.

146. Hill, E.V., and Aeberly, J.J.: "What About Ozone?," Heating and Ventilating, 31-34, 18 November; 29-33, 18 December; 37-40, 19 January; 33-35, 19 February; 36-37, 19 March, 1921-1922.

147. Hill, R.F.: "Effects of Illumination on Plaque Formation by Escherichia coli Infected with T_1 Bacteriophage," J Bact, 71:231-235, 1956.

148. Hodes, H.L.; Webster, L.T.; and Lavin, G.I.: "The Use of Ultraviolet Light in Preparing Non-Virulent Antirabies Vaccines," J Exptl Med, 72:437-444, 1940.

149. Hollaender, A.: "Abiotic and Sublethal Effects of Ultraviolet Radiation on Microorganisms," Aerobiology, Publication 17, AAAS, Washington, D.C., 1942. pp. 156-165.

150. Hollaender, A.: "Effects of Ultraviolet Radiation," Ann Rev Physiol, 8:1-16, 1946.

151. Hollaender, A.: Radiation Biology: Ultraviolet and Related Radiations, Vol. II, McGraw-Hill Book Company, Inc., New York, 1955.

152. Hollaender, A., and Claus, W.D.: "The Bactericidal Effect of Ultraviolet Radiation on Escherichia coli in Liquid Suspensions," J Gen Physiol, 19:753-765, 1936.

153. Hollaender, A., and Oliphant, J.W.: "The Inactivating Effect of Monochromatic Ultraviolet Radiation on Influenza Virus," J Bact, 48: 447-454, 1944.

154. Hollaender, A.; Jones, M.F.; and Jacobs, L.: "The Effects of Monochromatic Ultraviolet Radiation on Eggs of the Nematode Enterobius vermicularis. I. Quantitative Response," J Parasitol, 26:421-432, 1940.

155. Horsfall, F.K., Jr., and Bauer, J.H.: "Individual Isolation of Infected Animals in a Single Room," J Bact, 40:569-580, 1940.

156. Hosler, W.W.: "Germicidal Ultraviolet Tubes Kept Strong-Cobb Packaging Pure," Elec Prod Mag, 24:115, 1951.

157. Huntington, E.: Ozone Atmospheric-Effect on Animal Reproduction. Mainsprings of Civilization, J. Wiley and Sons, New York, 1945.

158. Hurwitz, C.; Rosano, C.L.; and Blattberg, B.: "A Test of the Validity of Reactivation of Bacteria," J Bact, 73:743-746, 1957.

159. Idoine, L.S.: Personal Communication, November 1958.

160. Idoine, L.S.; Matarrese, J.P.; and Lapedes, D.N.: "The Effect of Wave Lengths 2900A and Above Upon the Viability of Bacterial Aerosols," Bact Proc, p.142, 1957.

161. Jagger, J.: "Photoreactivation," Bact Revs, 22:99-142, 1958.

162. James, G.; Korns, R.F.; and Wright, A.W.: "Homologous Serum Jaundice Associated with the Use of Irradiated Plasma," J Amer Med Assoc, 144: 228-229, 1950.

163. Johnson, F.H.: "Effect of Electromagnetic Waves on Fungi," Phytopathology, 22:277-300, 1932.

164. Jones, M.F.; Jacobs, L.; and Hollaender, A.: "The Effects of Monochromatic Ultraviolet Radiation on Eggs of the Nematode Enterobius vermicularis. II. Sublethal Effects," J Parasitol, 26:435-445, 1940.

165. Jordon, E.O.; Carlson, A.J.; and Sawyer, W.A.: "The Bactericidal Deodorizing and Physiologic Properties of Ozone," J Amer Med Assoc, 61:1007-1012, 1913.

166. Kaye, S.: "The Use of Ethylene Oxide for the Sterilization of Hospital Equipment," J Lab Clin Med, 35:823-828, 1950.

167. Kelner, A.: "Effect of Visible Light on the Recovery of Streptomyces griseus Conidia from Ultraviolet Irradiation Injury," Proc Natl Acad Sci (U.S.), 35:73-79, 1949.

168. Kelner, A.: "Photoreactivation of Ultraviolet-Irradiated Escherichia coli with Special Reference to the Dose-Reduction Principle and to Ultraviolet-Induced Mutation," J Bact, 58:511-522, 1949.

169. Kelner, A.: "Action Spectra for Photoreactivation of Ultraviolet-Irradiated Escherichia coli and Streptomyces griseus," J Gen Physiol, 34:835-852, 1951.

170. Kessel, J.F.; Allison, D.K.; Moore, F.J.; and Kime, M.: "Comparison of Chlorine and Ozone as Virucidal Agents of Poliomyelitis Virus," Proc Soc Expt1 Biol Med, 53:71-73, 1943.

171. Kessel, J.F.; Allison, D.K.; Kime, M.; Quiros, M.; and Gloeckner, A.: "The Cysticidal Effects of C_1 and Ozone on Cysts of Endamoeba histolytica Together with a Comparative Study of Several Encystment Media," Amer J Trop Med, 24:177-183, 1944.

172. Knott, E.K., and Hancock, V.K.: "Irradiated Blood Transfusion in Treatment of Infections," Northwest Med, 33:200-204, 1934.

173. Koller, L.R.: "Bactericidal Effects of Ultraviolet Radiations Produced by Low Pressure Mercury Vapor Lamps," J Appl Phy, 10:624-630, 1939.

174. Koller, L.R.: Ultraviolet Radiation, John Wiley and Sons, Inc, New York, 1952.

175. Kovacs, R.: Electrotherapy and Light Therapy, 6th Edition, Lea and Febiger, Philadelphia, Pennsylvania, 1953.

176. Kraissi, C.J.; Cimiotti, J.G.; and Meleney, F.: "Considerations in the Use of Ultraviolet Radiation in Operating Rooms," Ann Surg, 111: 161-185, 1940.

177. Kupper, L.A.: "The Use of Ozone in Ventilation," J Ind Eng Chem, 6:353-356, 1914.

178. Latarjet, R., and Wahl, R.: "Precisions sur l'inactivation des bacteriophages par les rayons ultraviolettes," Ann Inst Pasteur, 71:336-339, 1945.

179. Laurell, G., and Ronge, H.: "Ultraviolet Air Disinfection in a Children's Hospital. A Technical and Bacteriological Study," Acta Paediatrica, 44:407-425, 1955.

180. Laurence, C.A., and Graikoski, J.: "Effect of Radiations on Microorganisms and Certain Biological Systems," University of Michigan Memorial-Phoenix Project, Progress Report 1, 1952. pp. 10-13.

181. Laurens, H.L.: "The Physiological Effects of Radiant Energy," Aerobiology, Publication 17, AAAS, Washington, D.C., 1942. p. 142.

182. Lea, C.H.: "Rancidity in Edible Fats," Department of Scientific and Industrial Research, Food Investigation, Special Report 46, His Majesty's Stationery Office, London, 1938.

183. Lea, D.E.: Action of Radiation on Living Cells, 2nd Edition, Cambridge University Press, Cambridge, 1955.

184. Levinson, S.O.; Milzer, A.; Shaughnessy, H.J.; Neal, J.J.; and Oppenheimer, F.: "A New Method for the Production of Potent Inactivated Vaccines with Ultraviolet Irradiation. II. Sterilization of Bacteria and Immunization with Rabies and St. Louis Encephalitis Vaccine," J Immunol, 50:317-329, 1945.

185. Luckiesh, M.: Applications of Germicidal, Erythemal and Infrared Energy, D. Van Nostrand Co., Inc., New York, 1946.

186. Luckiesh, M., and Holladay, L.L.: "Disinfecting Water by Means of Germicidal Lamps," Gen Elec Rev, 47:45-50, 1944.

187. Luckiesh, M., and Taylor, A.H.: "Radiant Energy from Fluorescent Lamps," Illum Eng, 40:77-88, 1945.

188. Luckiesh, M., and Taylor, A.H.: "Transmittance and Reflectance of Germicidal Energy," J Opt Soc Amer, 36:227-234, 1946.

189. Luckiesh, M.; Holladay, L.L.; and Taylor, A.H.: "Reaction of Untanned Human Skin to Ultraviolet Radiation," J Opt Soc Amer, 20:423-432, 1930.

190. Luckiesh, M.; Kerr, G.P.; and Knowles, T.: "Killing Bacteria in Water Under Pressure," Gen Elec Rev, 50:16-21, 1947.

191. Luckiesh, M.; Taylor, A.H.; and Knowles, T.: "Killing Airborne Respiratory Microorganisms with Germicidal Energy," J Franklin Inst, 244:267-290, 1947.

192. Luckiesh, M.; Taylor, A.H.; Knowles, T.; and Leppelmeier, E.T.: "Inactivation of Molds by Germicidal Ultraviolet Energy," J Franklin Inst, 248:311-325, 1949.

193. Luria, S.E., and Latarjet, R.: "Ultraviolet Irradiation of Bacteriophage During Intracellular Growth," J Bact, 53:149-163, 1947.

194. Lurie, M.B.: "Experimental Epidemiology of Tuberculosis. Airborne Contagion of Tuberculosis in an Animal Room," J Exptl Med, 51:743-751, 1930.

195. Lurie, M.B.: "Experimental Epidemiology of Tuberculosis. The Prevention of Natural Airborne Contagion of Tuberculosis in Rabbits by Ultraviolet Irradiation," J Exptl Med, 79:559-572, 1944.

196. Lurie, M.B.: "Experimental Airborne Tuberculosis," Amer J Med Assoc, 209:156-162, 1945.

197. Lurie, M.B.: "Control of Airborne Contagion of Tuberculosis," Amer J Nursing, 46:808-810, 1946.

198. MacVandiviere, H.; Sunkes, E.J.; and Smith, C.E.: "Experimental Data Preceding and Following Ultraviolet Installation at Battey State Tuberculosis Hospital." Presented before the laboratory section of the American Public Health Association, October 1949.

199. Magondeaux, B.R.C.: "Apparatus for Sterilizing Air by Ultraviolet Rays," French Patent 729,022, March 9, 1931.

200. Mallman, W.L., and Churchill, E.S.: "The Control of Microorganisms in Food Storage Rooms," Refrig Eng, 51:523-528, 1946.

201. Mashimo, T.: "A Method of Investigating the Action of Ultraviolet Rays on Bacteria," Mem Coll Sci, Kyoto Imp Univ, 4:1-11, 1919.

202. Matelsky, I.: "Germicidal Lamps in the Pharmaceutical Industry," Pharm Intern, 21:147, 1951.

203. Mayer, E., and Dworski, M.: "The Bactericidal Action of Ultraviolet Irradiations of Tubercle Bacilli," Amer Rev Tuberc, 26:105-111, 1932.

204. McCord, C.P., and Witheridge, W.N.: Odors, Physiology and Control, McGraw-Hill Book Co., Inc., New York, 1949.

205. McCoy, G.W.: "Accidental Psittacosis Infection Among the Personnel of the Hygenic Laboratory," Public Health Repts, 45:843-845, 1930.

206. McKhann, C.F.; Steeger, A.; and Long, A.P.: "Hospital Infections. I. A Survey of the Problem," Amer J Diseases Children, 55:579-599, 1938.

207. Medical Research Council: "Air Disinfection with Ultraviolet Irradiation. Its Effect on Illness Among School Children by the Air Hygiene Committee," Special Report Series 283, Her Majesty's Stationery Office, London, 1954.

208. Mefford, R., and Wyss, O.: "The Mutability of Bacillus anthracis Spores During Germination," J Bact, 61:357-364, 1951.

209. Meleney, F.L.: "Infection in Clean Operative Wounds. Nine Year Study," Surg Gynecol Obstet, 60:264-276, 1935.

210. Meyer, A.E.H., and Seitz, E.O.: Ultraviolette Strahlen, 2nd Edition, Walter de Gruyter and Co., Berlin , 1949.

211. Miller, S., and Ehrlich, R.: "Susceptibility to Respiratory Infections of Animals Exposed to Ozone. I. Susceptibility to Klebsiella pneumoniae," J Infectious Diseases, 103:2:145-149, 1958.

212. Miller, S.; Burkhardt, B.; Ehrlich, R.; and Peterson, R.J.: "Disinfection and Sterilization of Sewage by Ozone," International Ozone Conference, Chicago, Illinois, November 28-30, 1956.

213. Milzer, A.; Oppenheimer, F.; and Levinson, S.O.: "A New Method for the Production of Potent Inactivated Vaccines with Ultraviolet Irradiation. III. A Completely Inactivated Poliomyelitis Vaccine with the Lansing Strain in Mice," J Immunol, 50:331-340, 1945.

214. Mizuno, K.: "Studies on the Effect of Ultraviolet Rays Upon the Bacteriophage and Its Physico-Chemical Nature," Japan J Med Sci, VI. Bact and Parasitol, 1:52-87, 1929.

215. Moore, B., and Webster, T.A.: "Action of Light Rays on Organic Compounds, and the Photosynthesis of Organs from Inorganic Compounds in the Presence of Inorganic Colloids," Proc Roy Soc, London, 90:168-186, 1918.

216. Murphy, W.P., Jr., and Workman, W.G.: "Serum Hepatitis from Pooled Irradiated Dried Plasma," J Amer Med Assoc, 152:1421-1423, 1953.

217. Murray, R.; Oliphant, J.W.; Tripp, J.T.; Hampil, B.; Ratner, F.; Diefenbach, W.C.L.; and Geller, H.: "Effect of Ultraviolet Radiation on the Infectivity of Icterogenic Plasma," J Amer Med Assoc, 157: 8-14, 1955.

218. Nagy, R.: "Ultraviolet Lamps to Control Fungi," Food Industries, 19:928-929, 1947.

219. Nagy, R.: "Determination of Ozone," Unpublished report, April 10, 1952.

220. Nagy, R.: Personal Communications, June 1953, March 1954, and August 1954.

221. Nagy, R.: "A Resumé of the Bactericidal, Physiological and Chemical Action of Ozone," Westinghouse Electric Corporation, Research Dept., Bloomfield, New Jersey.

222. Nelson, J., and Matelsky, I.: "Rays Curb Bacteria, Boost Fruit Quality," Food Industries, 22:1722, 1950.

223. Newcomber, H.S.: "The Abiotic Action of Ultraviolet Light," J Exptl Med, 26:841-848, 1917.

224. Nicholson, E.: "Pasteurizing with Ultraviolet Gives Low Bacteria Count Milk," Food Industries, 19:1495-1496, 1615-1616, 1947.

225. Nielsen, R.A.: "Active Gases Associated with the Electrostatic Precipitator," Research Report R-94216-A, Westinghouse Electric Corporation, Bloomfield, New Jersey.

226. Nordberg, M.E.: "Ultraviolet-Transmitting Glasses for Mercury-Vapor Lamps," J Amer Ceram Soc, 30:174-179, 1947.

227. Odom, G.L.; Dratz, H.M.; and Kristoff, F.V.: "The Effect of Ultraviolet Radiation on the Exposed Brain. Experimental Study," Ann Surg, 130:68-75, 1949.

228. Ohlmuller, W.: "Uber die Einwirkung des Ozons auf Bacterien," Arb a d k Gsndhtsomte, Berlin, 8:229-251, 1892.

229. Oliphant, J.W., and Hollaender, A.: "Homologous Serum Jaundice. Experimental Inactivation of the Etiologic Agent in Serum by Ultraviolet Irradiation," Public Health Repts, 61:598-602, 1946.

230. Olsen, J.C., and Ulrich, W.H.: "Ozone in Ventilation," J Ind Eng Chem, 6:619-623, 1914.

231. Oppenheimer, F., and Levinson, S.O.: "A New Method for the Production of Potent Inactivated Vaccines with Ultraviolet Irradiation. I. Principles, Technic and Apparatus." Publication withheld by the Office of Scientific Research and Development.

232. Oppenoorth, W.F.F.: "Influence of Light on Sporulation of Brewing Yeasts," Nature, 178:992-993, 1956.

233. Paneth, F.A., and Edgar, J.L.: "Concentration and Measurement of Atmospheric Ozone," Nature, 142:112-113, 1938.

234. Paneth, F.A., and Gluckauf, E.: "Measurement of Atmospheric Ozone by a Quick Electrochemical Method," Nature, 147:614-615, 1941.

235. Perkins, J.E.; Bahlke, A.M.; and Silverman, H.F.: "Effect of Ultraviolet Irradiation of Classrooms on Spread of Measles in Large Rural Central Schools," Amer J Public Health, 37:529-537, 1947.

236. Phillips, G.B.; Novak, F.E.; and Alg, R.L.: "Portable Inexpensive Plastic Safety Hood for Bacteriologists," Appl Microbiol, 3:216-217, 1955.

237. Phillips, G.B.; Jemski, J.V.; and Brant, H.G.: "Cross Infection Among Animals Challenged with Bacillus anthracis," J Infectious Diseases, 99:222-226, 1956.

238. Phillips, G.B.; Broadwater, G.C.; Reitman, M.; and Alg, R.L.: "Cross Infections Among Brucella Infected Guinea Pigs," J Infectious Diseases, 99:56-59, 1956.

239. Polson, A., and Dent, J.: "The Rate of Inactivation by Ultraviolet Irradiation as a Means of Distinguishing Antigenically Different Strains of African Horse-Sickness Virus," Brit J Expti Path, 31:1-4, 1950.

240. Post, M.H., Jr.: "Prevention of Infection in Ophthalmic Surgery. Further Studies," Amer J Ophthalmol, 36:1091-1099, 1953.

241. Post, M.H., Jr.: "Prevention of Infection in Ophthalmic Surgery. Protection of Instruments from Dust-Borne Contamination and Sterilization of Certain Solutions by Ultraviolet Radiations," Amer J Ophthalmol, 36:1258-1264, 1953.

242. Powell, S.T.: "Ozone for Ventilation," Met Chem Eng, 13:975-977, 1915.

243. Ramsey, G.B., and Bailey, A.A.: "Effects of Ultraviolet Radiation Upon Sporulation in Macrosporium and Fusarium," Botan Gaz, 89:113-136, 1930.

244. Reitman, M., and Wedum, A.G.: "Microbiological Safety," Public Health Repts, 71:659-665, 1956.

245. Rentschler, H.C., and Nagy, R.: "Advantages of Bactericidal Ultraviolet Radiation in Air Conditioning Systems," Heating, Piping and Air Conditioning, 12:127-130, 1940.

246. Rentschler, H.C., and Nagy, R.: "Bactericidal Action of Ultraviolet Radiation on Airborne Organisms," J Bact, 44:85-94, 1942.

247. Rentschler, H.C.; Nagy, R.; and Mouvomseff, G.: "Bactericidal Effect of Ultraviolet Radiation," J Bact, 41:745-774, 1941.

248. Ricks, H.C.; Cortelyou, J.R.; Labecki, T.D.; McWhinnie, M.A.; Underwood, F.J.; Semrad, J.E.; and Reeves, G.R.: "Practical Application of Ultraviolet Radiation in Purification of Naturally Contaminated Water," Amer J Public Health, 45:1275-1281, 1945.

249. Ridenour, G.M., and Ingols, R.S.: "Inactivation of Poliomyelitis Virus by Free Chlorine," Amer J Public Health, 36:639-644, 1946.

250. Rieck, A.F., and Carlson, S.D.: "Photorecovery from the Effects of Ultraviolet Radiations in the Albino Mouse," J Cellular Comp Physiol, 46:301-305, 1955.

251. Riley, R.L.: "Aerial Dissemination of Pulmonary Tuberculosis," Amer Rev Tuberc and Pulmonary Diseases, 76:931-941, 1957.

252. Riley, R.L.; Wells, W.F.; Mills, C.C.; Nyka, W.; and McLean, R.L.: "Air Hygiene in Tuberculosis. Quantitative Studies of Infectivity and Control in a Pilot Ward," Amer Rev Tuberc and Pulmonary Diseases, 75:420-431, 1957.

253. Robbins, F.C., and Rustigan, R.: "Q Fever in the Mediterranean Area. Report of Its Occurrence in Allied Troops. IV. A Laboratory Outbreak," Amer J Hyg, 44:64-71, 1946.

254. Robertson, E.C., and Doyle, M.E.: "On the Control of Airborne Bacteria in Operating Rooms and Hospital Wards," Ann Surg, 111:491-497, 1940.

255. Robertson, E.C.; Doyle, M.E.; and Tisdall, F.F.: "Use of Ultraviolet Radiation in Reduction of Respiratory Cross Infection," J Amer Med Assoc, 121:908-914, 1943.

256. Robertson, E.C.: Doyle, M.E.; Tisdall, F.F.; Koller, L.R.; and Ward, F.S.: "Air Contamination and Air Sterilization," Amer J Diseases Children, 58:1023-1037, 1939.

257. Romig, W.R., and Wyss, O.: "Some Effects of Ultraviolet Radiation on Sporulating Cultures of Bacillus cereus," J Bact, 74:386-391, 1957.

258. Ronge, H.E.: Ultraviolet Irradiation with Artificial Illumination, Almqvist and Wiksells Boktryckeri, Uppsala, Sweden (in English), 1948.

259. Rooks, R.: "Bacterial Lamp Conjunctivitis," J Iowa State Med Soc, 35:141, 1945.

260. Rosebury, T.: Experimental Airborne Infection, Williams and Wilkens Company, Baltimore, Maryland, 1947.

261. Rosenstern, I.: "Observations on the Control of Respiratory Contagion in the Cradle," Aerobiology, Publication 17, AAAS, Washington, D.C., 1942. p. 242.

262. Rusch, H.P.; Kline, B.E.; and Baumann, C.A.: "Carcinogenesis by Ultraviolet Rays with Reference to Wave Length and Energy," Arch Pathol, 31:135-146, 1941.

263. Sarber, R.W.; Nungester, W.J.; and Stimpert, F.D.: "Immunization Studies with Irradiated Tuberculosis Vaccines," Amer Rev Tuberc, 62: 418-427, 1950.

264. Sawyer, W.A.; Beckwith, H.L.; and Skolfield, E.M.: "The Alleged Purification of Air by the Ozone Machine," J Amer Med Assoc, 61:1013-1015, 1913.

265. Schneckenberg, E.: "Physiologische Versuche mit Ozonluft, Vorwiegerd bei Ozongehalten um 0.0001% wie bie Ozonventilation Sanlagen," Gesundh Ingr, 35:965-970, 1912.

266. Sharp, D.G.: "A Quantitative Method of Determining the Lethal Effect of Ultraviolet Light on Bacteria Suspended in Air," J Bact, 35:569-599, 1938.

267. Sharp, D.G.: "The Lethal Action of Short Ultraviolet Rays on Several Common Pathogenic Bacteria," J Bact, 37:447-460, 1939.

268. Sharp, D.G.: "The Effects of Ultraviolet Light on Bacteria Suspended in Air," J Bact, 39:535-547, 1940.

269. Silverman, L.: "Industrial Air Sampling and Analysis," Chemistry and Toxicology Series, Bulletin 1, Industrial Hygiene Foundation, Pittsburgh, Pennsylvania, 1947. pp. 1-64.

270. Smith, W.W., and Bodkin, R.E.: "The Influence of Hydrogen Ion Concentration on the Bactericidal Action of Ozone and Chlorine," J Bact, 47:445, 1944.

271. Smolens, J., and Stokes, J., Jr.: "Combined Use of Ultraviolet Irradiation and Beta-Propiolactone Sterilization of Sera Infected with a Virus," Proc Soc Exptl Biol Med, 86:536-539, 1954.

272. Snell, F.D., and Snell, C.T.: Colorimetric Method of Analysis, 3rd Edition, Vol. 3, D. Van Nostrand Co., Inc., New York, 1954. p. 886.

273. Sollman, T.H.: A Manual of Pharmacology and Its Applications to Therapeutics and Toxicology, W.B. Saunders Co., Philadelphia, Pennsylvania, 1948.

274. Spector, W.S.: Handbook of Biological Data, WADC Technical Report 56-273, W. B. Saunders Co., Philadelphia, Pennsylvania, October 1956.

275. Stanford Research Institute: "The Smog Problem in Los Angeles County." Distributed by Western Oil and Gas Association, Los Angeles 14, California, January 1944.

276. Stuy, J.H.: "Photoreactivation of Ultraviolet Inactivated Bacteria," Biochem et Biophys Acta, 17:206-211, 1955.

277. Stuy, J.H.: "Studies on the Mechanism of Radiation Inactivation of Microorganisms. III. Inactivation of Germinating Spores of Bacillus cereus," Biochem et Biophys Acta, 22:241-246, 1956.

278. Sulkin, E.S., and Pike, R.M.: "Survey of Laboratory Infections," Amer J Public Health, 41:769-781, 1951.

279. Sutton, W.S.: "Irradiation of Cheese Molds and Bacteriophage by Ultraviolet Light," J Australian Inst Agric Sci, 7:67-73, 1941.

280. Taylor, A.H.: "Measuring Germicidal Energy," Gen Elec Rev, 47:53, 1944.

281. Taylor, A.H., and Haynes, H.: "New Meters for Germicidal Energy," Gen Elec Rev, 50:10:27-29, 1947.

282. Taylor, A.R.; Sharp, D.G.; Beard, D.; Finkelstein, H.; and Beard, J.W.: "Influence of Ultraviolet Light on Equine Encelphalomyelitis Virus Protein (Eastern Strain)," J Infectious Diseases, 69:224-231, 1941.

283. Thorp, C.E.: "Starch-Iodine Method of Ozone Analysis," Ind Eng Chem, 12:209, 1940.

284. Thorp, C.E.: "Influence of Nitrogen Oxides on the Toxicity of Ozone," Chem Eng News, 19:686-688, 1941.

285. Thorp, C.E.: "The Toxicity of Ozone. A Report and Bibliography," Ind Med and Surg, 19:45-57, 1950.

286. Thorp, C.E.: Personal Communication, Armour Research Foundation, October 1951.

287. Thorp, C.E.: Bibliography of Ozone Technology. Vol. I. Analytical Procedures and Patent Index. Vol. II. Physical and Pharmacological Properties, Armour Research Foundation, Chicago, Illinois, 1954.

288. Tomlinson, A.J.H.: "Infected Airborne Particles Liberated on Opening Screw-Capped Bottles," Brit Med J, 5035:15-17, 1957.

289. Torricelli, A.: "Sterilization of Containers in the Food Industry," International Ozone Conference, Chicago, Illinois, November 28-30, 1956.

290. Trotskii, V.L.; Sviridova, T.A.; and Super, L.P.: "The Action of Ultraviolet Light on the Antigenic, Toxic and Immunization Characteristics of Bacteria," J Microbiol Epidemiol Immunobiol, USSR, 15:519-531, 1935.

291. Van Atta, F.A.: Personal Communication, National Safety Council, August 16, 1951.

292. Van Ermengem, E.: "De la sterilisation des eaux par l'ozone," Ann Inst Pasteur, 9:673-709, 1895.

293. Von Kupffer, L.A.: "Verwendurg des Ozons bei des Luftung, Gesundh Ingr, 36:605-615, 1913.

294. Voogd, J., and Daams, J.: "Inactivation of Bacteria by Ultraviolet Radiation," Phillips Tech Rev, 12:111-119, 1950.

295. Vosmaer, A.: "Applications of Ozone," J Ind Eng Chem, 6:229-232, 1914.

296. Ward, H.M.: "Experiments on the Action of Light on Bacillus anthracis," Proc Roy Soc, London, 52:393-400, 1892.

297. Ward, H.M.: "The Action of Light on Bacteria," Proc Roy Soc, London, 54:472-475, 1893.

298. Watson, R.D.: "Some Factors Influencing the Toxicity of Ozone to Fungi in Cold Storage," Refrig Eng, 46:103-106, 1943.

299. Weaver, E.R.: "The Use of Ozone in Diminishing Perception of Odors in Occupied Buildings," Report 1328, U.S. Dept of Commerce, National Bureau of Standards, 1951.

300. Wedum, A.G.: "Bacteriological Safety," Amer J Public Health, 43:1428-1437, 1953.

301. Wedum, A.G.: "Protecting Laboratory Workers from Accidental Infections," Committee Report, Laboratory Section, Committee on Laboratory Infections and Accidents." Presented at the 84th Annual Meeting of the American Public Health Association, Atlantic City, New Jersey, November 13, 1956.

302. Weinstein, I.: "Quantitative Biological Effects of Monochromatic Ultraviolet Light," J Optical Soc Amer, 20:433-456, 1930.

303. Welch, H.: "The Effect of Ultraviolet Light on Molds, Toxins and Filtrates," Preventive Med, 4:295-330, 1930.

304. Wells, W.F.: "Studies on Airborne Infection," Science, 92:457-458, 1940.

305. Wells, W.F.: "Bactericidal Irradiation of Air," J Franklin Inst, 229:347-372, 1940.

306. Wells, W.F.: "Radiant Disinfection of Air," Arch Phys Therapy, 23: 143-148, 1942.

307. Wells, W.F., and Brown, H.W.: "Recovery of Influenza Virus Suspended in Air and Its Destruction by Ultraviolet Radiation," Amer J Hyg, 24:407-413, 1936.

308. Wells, W.F., and Lurie, M.B.: "Experimental Airborne Disease. Quantitative Natural Respiratory Contagion of Tuberculosis," Amer J Hyg, (Section B), 34:21-40, 1941.

309. Wells, M.W., and Holla, W.A.: "Ventilation in the Flow of Measles and Chickenpox Through a Community," J Amer Med Assoc, 142:1337-1344, 1950.

310. Westinghouse Electric Corporation: "Phototubes," Ultraviolet Data Sheet 86-0800, Westinghouse Electric Corporation, Bloomfield, New Jersey, 1944.

311. Westinghouse Electric Corporation: "Ultraviolet Meter SM-200," Data Sheet 86-099, Westinghouse Electric Corporation, Bloomfield, New Jersey, 1948.

312. Westinghouse Electric Corporation: Engineering Booklet ASC-144, Westinghouse Electric Corporation, Bloomfield, New Jersey, 1951.

313. Westinghouse Electric Corporation: Data Sheet ASC-152, Westinghouse Electric Corporation, Bloomfield, New Jersey, 1957.

314. Westinghouse Electric Corporation: Bulletin ASC-22, Westinghouse Electric Corporation, Bloomfield, New Jersey.

315. Westinghouse Electric Corporation: Bulletin ASC-170 Revised, Westinghouse Electric Corporation, Bloomfield, New Jersey.

316. Wheeler, S.M.; Ingraham, H.S.; Hollaender, A.; Lill, N.D.; Gershon-Cohen, J.; and Brown, E.W.: "Ultraviolet Light Control of Airborne Infections in a Naval Training Center," Amer J Public Health, 35: 457-468, 1945.

317. Whitehead, H.R., and Hunter, G.J.E.: "Bacteriophage Infection in Cheese Manufacture," J Dairy Research, 14:64-80, 1945.

318. Whisler, B.A.: "The Efficacy of Ultraviolet Light Sources in Killing Bacteria Suspended in Air," Iowa State College J Sci, 14:215-231, 1940.

319. Whitwell, E.; Taylor, P.F.; and Oliver, A.J.: "Hazards to Laboratory Staff in Centrifuging Screw-Capped Containers," J Clin Pathol, 10:88-91, 1957.

320. Witheridge, W.N., and Yaglou, C.P.: "Ozone in Ventilation," Ice and Refrig, 97:78, 1939.

321. Witkin, E.M.: "Nuclear Segregation and the Delayed Appearance of Induced Mutations in Escherichia coli," Cold Spring Harbor Symposia Quant Biol, 16:357-371, 1951.

322. Wolf, A.M.; Mason, J.; Fitzpatrick, W.; Schwartz, S.C.; and Levinson, S.O.: "Ultraviolet Irradiation of Human Plasma to Control Homologous Serum Jaundice," J Amer Med Assoc, 135:476-477, 1947.

323. Woodhall, B.; Neill, R.G.; and Dratz, H.M.: "Ultraviolet Radiation as an Adjunct in Control of Post-Operative Neurosurgical Infection. II. Clinical Experience 1938-1948," Ann Surg, 129:820-825, 1949.

324. Woodridge, F.V.: "The Bactericidal Deodorizing and Physiological Effect of Ozone," Eng News, 71:778-779, 1914.

325. Woodside, E.E., and Kocholaty, W.: "A Comparison of Ultraviolet Sensitivity and Photoreactivation Phenomena in Radiation Resistant and Sensitive Strains of Escherichia coli," Army Medical Research Laboratory, Report 142, 1954.

326. Wyckoff, R.W.G.: "The Killing of Colon Bacilli by Ultraviolet Light," J Gen Physiol, 15:351-361, 1932.

327. Zelle, M.R., and Hollaender, A.: "Monochromatic Ultraviolet Action Spectra and Quantum Yields for Inactivation of T_1 and T_2 Escherichia coli Bacteriophage," J Bact, 68:210-215, 1954.

328. Zoeller, G.: "Action des rayons ultraviolet sur une souche de bacteriophage," Compt Rend Soc Biol, 89:860-861, 1923.

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